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THE GERMINATION OF FUNGOUS SPORES IN RELATION TO CONTROLLED HUMIDITY

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(Accepted for publication March 11, 1942)

Water, either liquid or vapor, has long been recognized as essential to the germination of spores of most pathogenic fungi. Furthermore, high atmospheric humidities are known to favor the initiation of infection of plants by fungous pathogens and the subsequent development of epiphytotics of plant diseases. However, fully convincing experimental data on the relation of high relative humidities to the germination of spores of most phytopathogenic fungi have been lacking, because of the difficulties of controlling the experimental conditions with such accuracy as to preclude the possible intervention of minute amounts of liquid water. The investigation reported herein was undertaken (1) to develop an accurate method for spore germination experiments at high relative humidities, and (2) to determine the relation of relative humidity to the germination of spores of certain phytopathogenic fungi.

LITERATURE REVIEW

Before humidity relations of fungi could be studied, suitable methods for controlling relative humidities had to be devised. In 1895 Lesage (18) made use of the vapor-pressure values of different concentrations of NaCl in one of the earliest attempts to determine the effect of relative humidity upon the germination of spores of *Penicillium glaucum*. The method for studying the humidity relations of fungi in culture by the use of aqueous solutions of H_2SO_4 was first described by Stevens (27) and further elaborated by Wilson (33). A laboratory method for maintaining constant humidity by means of saturated solutions of various chemicals was given by Spencer (26). Sweetman (29), in investigating the use of saturated solutions for the control of relative humidity in small closed containers, found that humidities obtained with certain saturated solutions did not change within 6 months. Critical discussions of the use of saturated salt solutions and of aqueous solutions of KOH, $CaCl_2 \cdot 6H_2O$, and H_2SO_4 to control the relative humidity have been presented by Hopp (16), Zwölfer (35), and others. Shaw (22) calculated from osmotic pressure values the relative humidities that solutions of sucrose of various weight-normal concentrations would provide at 25° C. Vernon and Whitby (32) pointed out that air, bubbled through a bottle full of water, is not necessarily saturated and that the method of controlling the humidity by mixing desiccated air with such presumably saturated air is faulty. One of the disadvantages of using H_2SO_4 is that SO_2 (35) or SO_3 (30) may be

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given off, especially if any organic material or particles drop into the solution.

Many observations and experiments on the relation of relative humidity to the germination of fungous spores have been made, chiefly, in conjunction with other lines of work, such as infection of plants, decay or deterioration of food products, and mildewing of textiles and book bindings. The data on the relation of relative humidity to spore germination in reports of such investigations cannot, in general, be considered highly accurate, because the conditions of the tests were not closely controlled, or, in many cases, they were not even stated. Such results may have been satisfactory for the purposes sought by the various investigators, but they can be considered merely indicative of the exact relation of humidity to the germination of fungous spores. In order to evaluate such data it is highly desirable to know the maximum fluctuation in the "constant" temperature at which tests are made.

Contact with water has been reported necessary to germination of all types of spores of many rust fungi; some experimental tests, however, have indicated that germination can occur in atmospheres just below saturation. Blackman (2) reported that teliospores of *Puccinia graminis, Phragmidium rubi,* and *Uromyces fabae* in a moist atmosphere germinated and formed normal sporidia, whereas, in hanging drops of water, spores produced long germ tubes that formed a few abnormal sporidia if the tubes came in contact with the air. Hirt (15) observed germination of teliospores of *Cronartium ribicola* on Ribes leaves and the formation and discharge of sporidia at relative humidities from 96 to 100 per cent. Sporidia of *Cronartium ribicola,* incubated on strips of cellophane for 48 hours at relative humidities ranging from 94 to 100 per cent, germinated abundantly at a humidity of 100 per cent, commonly at 99 per cent, and rarely at 97 per cent. Doran (8) stated that the aeciospores of *Gymnosporangium clavipes* germinated well on a dry slide in moist air. Smith (24) reported germination of urediospores of *Puccinia sorghi* on a dry substratum at relative humidities from 97.5 to 100 per cent. Stock (28) found that urediospores of *Puccinia triticina, P. dispersa, P. coronifera,* and *P. graminis* on dry slides failed to germinate at relative humidities below 99 per cent. Hemmi and Abe (14) stated that the average germination of urediospores of *Puccinia glumarum, P. triticina,* and *P. lolli* on dry slides at relative humidities of 100, 99, and 95 per cent, respectively, ranged between 12 and 40, 1.5 and 16, 0 and 0.5 per cent.

That conidia of some powdery mildew fungi would germinate on glass slides or leaves in moist air was mentioned as early as 1884 (25). Corner (7) mentioned that conidia of *Erysiphe graminis, Podosphaera leucotricha, Sphaerotheca pannosa, E. cichoracearum,* and *Oidium euonymi-japonici,* when immersed in water, produced a short germ tube or, commonly, none at all, whereas on dry glass in a saturated atmosphere they germinated readily. Berwith (1) concluded that a high atmospheric humidity was essential for the germination of the conidia of the apple powdery mildew fungus. Dundas (9) found that spores of *Erysiphe polygoni* from bean would ger-

minate on dry slides at ordinary room humidity, as well as on water. Yarwood (34) reported that at favorable temperatures conidia of *Erysiphe polygoni* from red clover or bean germinated at relative humidities of 100 to approximately 0 per cent. Hashioka (13) reported that conidia of *Sphaerotheca fuliginea* germinated only in the saturated condition and were unable to germinate in relative humidities below 99 per cent. Longrée (19) made a careful and thorough study of the effect of temperature and relative humidity on the germination of conidia of *Sphaerotheca pannosa* var. *rosae*. In germination tests with conidia dusted on dry glass slides she experienced great difficulties in obtaining relative humidities close to 100 per cent without getting condensation. She accomplished it by means of a special humidity-control chamber in which temperature, and relative humidity, could be strictly controlled. The germination was excellent at a relative humidity of about 97 to about 99 per cent, somewhat lower at 99.8 to 100 per cent, very low at 95 per cent or below, and zero below 75 per cent. Germination of conidia dusted on leaves of rose shoots kept in a controlled environment was reduced as the atmospheric humidity of the environment was decreased; but, in general, spores on the surfaces of leaves germinated in an apparently very dry atmosphere. Brodie and Neufeld (3) reported germination of conidia of *Erysiphe polygoni* from *Delphinium* and from *Polygonum aviculare* and of *Erysiphe graminis* from *Poa pratensis* through a range of relative humidity from approximately zero to 100 per cent.

Guba (12) found that at least a relative humidity of 95 per cent was required for germination of the conidia of *Cladosporium fulvum* on dry glass.

Chowdhury (4, 5) attempted to determine the relation of relative humidity to the germination of spores of certain Indian fungi. Spores were placed on dry cellophane squares, previously soaked in 1 per cent glucose solution. The squares were then suspended over NaCl or H_2SO_4 solutions in stoppered bottles. His results indicated that the minimum relative humidity at which germination occurred was 90 per cent with spores of *Acrothecium penniseti*, *Alternaria brassicae*, and *Cladosporium herbarum;* 91 per cent with spores of *Helminthosporium frumentacei;* 93.9 per cent with spores of *Gloesporium tabernaemontanae*, and *Phyllosticta cajani;* and 95 per cent with spores of *Colletotrichum falcatum*, *C. lindemuthianum*, and *C. graminicolum*.

Tomkins (31), in tests at "known temperatures," observed spores of *Alternaria citri* germinating at relative humidities of 90.9 to 100 per cent. He concluded that the influence of the nature of the solid surfaces supporting the spores could be neglected, since the average lengths of germ tubes from spores on quartz, glass, Canada balsam, dammar, or shellac did not show any consistent differences.

Groom and Panisset (11) reported a minimum relative humidity of 81 per cent for germination of the spores of *Penicillium chrysogenum*.

Galloway (10) studied the moisture requirements of about 40 species of

the more common mold fungi in reference to mildew in textiles. Spores were placed on dry squares of cellophane that had been soaked in dilute wort of 1 per cent maltose content. The squares were suspended over $CaCl_2$ solutions in stoppered bottles. On such nutritive substrata the lowest relative humidity at which germination occurred was between 75 and 90 per cent for different species of *Aspergillus*, 80 and 85 for species of *Penicillium*, 85 and 90 per cent for *Trichoderma*, 90 per cent for *Stemphylium*, *Cladosporium*, *Acrothecium*, or *Stachybotrys*, and 95 per cent for *Thielaviopsis*.

METHODS AND MATERIALS

Saturated aqueous salt or acid solutions, aqueous sucrose solutions, anhydrous $CaCl_2$, and redistilled water were used to control the relative humidity within sealed 1-quart glass fruit jars used as humidity chambers. Salts or oxalic acid of analytical reagent grade, C.P. analyzed sucrose, and redistilled water from a block-tin still were used in preparation of control solutions (Table 1). The chambers were thoroughly cleaned and rinsed with

TABLE 1.—*Composition and concentration of solutions used to provide the stated theoretical relative humidities within sealed glass chambers at 20° C.*

Material used	Concentration of solution	Theoretical relative humidity[a]
		Per cent
Redistilled water	.....	100.0
Sucrose	0.10 molal	99.85
Sucrose	0.20 "	99.62
Sucrose	0.30 "	99.43
Sucrose	0.40 "	99.24
Sucrose	0.50 "	99.04
Sucrose	0.60 "	98.86
Sucrose	0.70 "	98.65
Sucrose	0.80 "	98.45
Sucrose	0.90 "	98.24
Sucrose	1.00 "	98.03
$H_2C_2O_4 \cdot 2H_2O$[b]	Saturated[c]	96.0
$Na_2HPO_4 \cdot 12H_2O$	"	95.0
$Na_2SO_4 \cdot 10H_2O$	"	93.0
K_2HPO_4	"	92.0
K_2CrO_4	"	88.0
$(NH_4)_2SO_4$	"	81.0
$Mg(C_2H_3O_2)_2 \cdot 4H_2O$	"	65.0
$Ca(NO_3)_2 \cdot 4H_2O$	"	55.0
$CaCl_2 \cdot 6H_2O$	"	32.0
$KC_2H_3O_2$	"	20.0
$CaCl_2$ (anhydrous)	.....	0.0

[a] Calculations for sucrose solutions based on data from Morse, *et al.* (20) and data for saturated solutions taken from Spencer (26) except as indicated in [b].

[b] Based on Obermiller's (21) and Shaw's (22) measurements.

[c] An excess of solid phase was maintained in contact with the liquid phase of each saturated solution.

redistilled water. The rubber gaskets for the chambers were washed in CS_2 for 10 minutes and dried at least 24 hours before they were used to remove any sulphur present on their surface. The amount of solution, or water, used in each chamber was 250 cc. In the saturated solutions (26) an excess

of the solid phase was always maintained. The theoretical relative humidities that various molal solutions of sucrose would provide in closed chambers were calculated by dividing the vapor pressures of the sucrose solutions by the vapor pressure of water at 20° C. The vapor pressures of the sucrose solutions were calculated from the osmotic pressure values (27).

After adding the required amount of sucrose and 230 cc. of redistilled water to each of the humidity chambers, they were sealed and steamed at

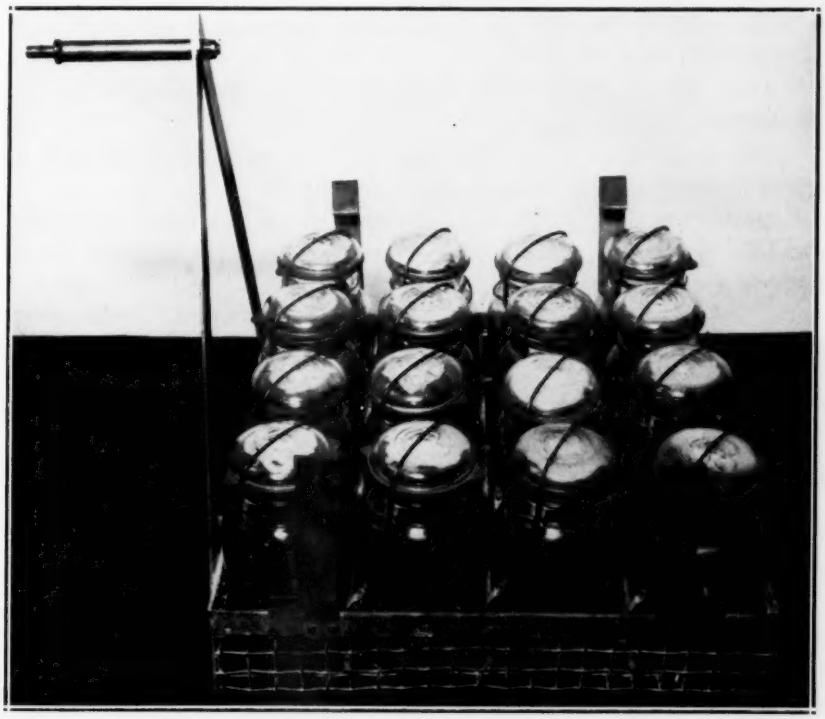

FIG. 1. Wire-mesh basket containing humidity chambers.

70° C. for 10 minutes to inactivate enzymes possibly present in the sucrose. After the chambers had cooled, 50 mg. of $K_2Cr_4O_7$, contained in 20 cc. of solution, were added to the sucrose solution in each chamber to prevent growth of organisms (6). Calculations showed that the $K_2Cr_4O_7$, assuming complete ionization, would theoretically lower the relative humidity within the chambers by 0.0003 per cent.

All experiments were made for 24, 48, or 72 hours in a water bath whose temperature was thermostatically controlled at 20° ± 0.02° C. During each series the temperature of the water bath, as indicated on a Beckmann thermometer that could be read to 0.01° C., was recorded at frequent intervals. The temperature of the water bath also was continuously recorded by means of a soil thermograph sensitive to changes of 0.2° C. or more. The room in which the experimental apparatus was assembled was kept at a temperature of 18°–20° C. and a relative humidity between 30 and 50 per cent.

To minimize stratification of the atmosphere or the solutions within the chambers the apparatus shown in figure 1 was used. The wire-mesh basket held 16 humidity chambers submerged in the water bath. The basket, on a brass rod support at its middle, was rocked to an angle of 17 degrees at a rate of 6 times a minute by means of a mechanical rocker arm connected to a gear reducer. An electric motor continuously operated the gear reducer and the water-bath agitator. The gentle rocking of the chambers caused no splashing of the solution within them.

Each series was maintained in the dark, exclusive of the short periods of diffuse light from the heating unit and the periods of several hours a day when a 150-watt electric bulb was used in the room in order that the necessary observations and experimental work could be performed. The exposure of the spores to rather diffuse light during these periods is believed to have been an insignificant factor affecting germination.

Experiments were made with conidia of *Sclerotinia fructicola* (Wint.) Rehm; ascospores and condia of *Venturia inaequalis* (Cke.) Wint.; urediospores of *Puccinia coronata* (Pers.) Cda., *P. graminis tritici* Eriks. and Henn., and *P. graminis avenae* Eriks. and Henn.; chlamydospores of *Ustilago hordei* (Pers.) K. and S. and *U. nuda* (Jens.) K. and S.; conidia of *Erysiphe polygoni* DC. from red clover (*Trifolium pratense* L.), cabbage (*Brassica oleracea* L.), and evening primrose (*Oenothera biennis* L.); and conidia of *E. graminis hordei* Marchal from barley (*Hordeum vulgare* L.) Precautions were taken to use as uniform spore material as was feasible.

Spores on or in drops of redistilled water on several clean cover glasses on glass slides in Petri-dish moist chambers at room temperature served as controls in each series.

Spores were dusted, brushed, or discharged naturally over the glass, paraffin, quartz, or leaf surfaces that served as spore-carriers (Fig. 2). Each carrier was then attached with paraffin to one end of a small glass rod. The other end of the rod was heated and imbedded in paraffin inside one of 4 Van Tieghem rings that had been sealed with paraffin to the inner part of a glass top of a humidity chamber. As many as 8 spore carriers could be attached to a single top within 2 minutes. This top was then substituted for one on a chamber in equilibrium with the water bath. The entire operation of removing a chamber, drying, making the substitution, and returning the chamber could be completed within 30 seconds.

Different solid substances were used as spore-carriers to determine (1) whether the tendency for condensation of water vapor on them varies to a degree sufficient to cause differences in germination at high relative humidities, or (2) whether differential effects might be exerted by minute amounts of soluble materials emanating from the spore-carriers. Non-nutritive spore-carriers were used, because the presence of extraneous materials on their surface probably would affect the relative humidity surrounding the spores. The glass surfaces were those of dry cover glasses that had been thoroughly cleaned in hot acid cleaning solution, rinsed several times in dis-

tilled water, placed over night in redistilled water, and dried with clean cheesecloth. The paraffin surfaces were those of cover glasses, thinly coated with imported white filtered paraffin with a high melting point, 68°–72° C. Prior to its use, the paraffin was kept at 85° C. for two weeks in order to rid it of any possible volatile materials. Clean pieces of fused or ground quartz were used in a limited number of series of experiments. The ventral leaf surfaces used in one series were portions of a large uninjured mature leaf from a potted Fameuse apple tree growing in the greenhouse. Disks 2.5 cm. in diameter were cut from the leaf with a cork borer. A disk was placed

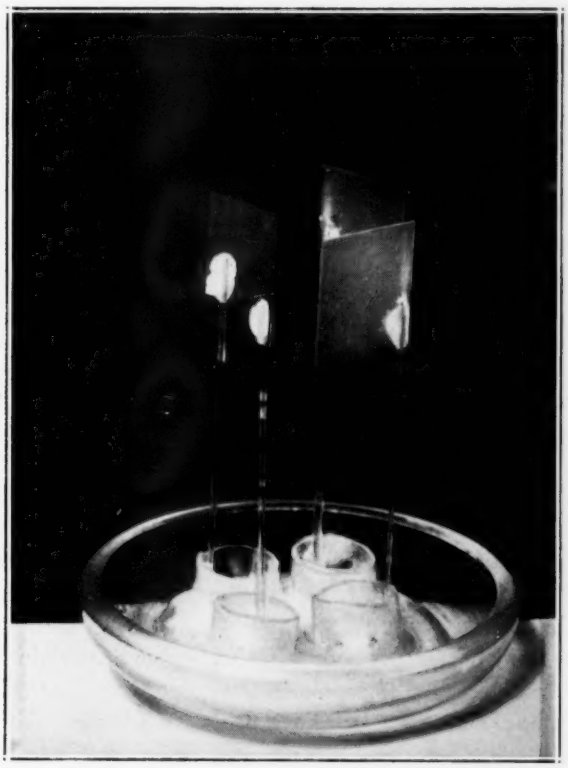

FIG. 2. Spore carriers attached by means of glass rods to the top of a humidity chamber.

on each of several cover glasses with the dorsal surface adjacent to the glass. Paraffin was used to seal the margin of the disk to the surface of a cover glass. After 24 hours in the chambers the disks were cleared in a solution of glacial acetic acid-ethyl alcohol. Care was taken to avoid dislodging any of the spores during the clearing; they, however, adhered tenaciously and were not easily dislodged.

To facilitate taking counts and to kill the spores at the end of each test, the spores usually were stained with aqueous crystal violet solution or with cotton blue in lactophenol. The spore carriers were mounted on slides,

using a rosin-lanolin mixture (23) for sealing the margins. Spores carefully mounted in such manner remained in satisfactory condition for many months.

Experimental data were expressed in percentage germination and in length of germ tube. Counts of the number of germinated and nongerminated spores in 3 to 10, usually 5, random samples of 100 spores each were made on every spore-carrier. Replicate spore carriers of each type of surface and replicate chambers at each relative humidity were usually used in 2 to 8 series with spores of each fungus. Therefore, the total number of spores of a fungus that was counted at each relative humidity on the glass, paraffin, quartz, or leaf surface ranged between 300 and 50,000. Measurements of the length of germ tubes from 25 to 150 germinated spores taken at random were made at the various relative humidities in several series with most fungi used.

EXPERIMENTAL RESULTS

Conidia of Sclerotinia fructicola

Colonies of a monoconidial isolate of *Sclerotinia fructicola* were grown on a malt-agar medium in Petri dishes at 20° C. Tufts of conidia under 4 days of age were touched lightly with a camel-hair brush that had the hairs clipped short and of the same length. Extreme care was taken to avoid touching the surface of the agar medium with the brush. The spores adhering to the brush were dusted or brushed lightly over glass, paraffin, or quartz spore carriers used in tests at high relative humidities for periods as long as 72 hours.

The mean germination of conidia in redistilled water after 4 hours was 12 per cent and after 48 hours 87 per cent, the means in the different series ranging between 65 and 97 per cent. The mean length of germ tubes from conidia in water after 3, 4, 6, 7½, 11, 16, and 22 hours, respectively, was 25, 40, 72, 97, 148, 305, and 474 μ.

Single conidia on dry glass or paraffin did not germinate at relative humidities of 100 per cent or below in 2 experimental series for 24 hours, 3 for 48 hours, and 1 for 72 hours, respectively. Likewise, single condia on quartz at relative humidities of 100 per cent or below for 48 hours failed to germinate. In a few rare instances, where a little agar medium had been carried by the brush to the surfaces of the spore carriers, a few conidia germinated at high relative humidities.

Ascospores of Venturia inaequalis

Ascospores of *Venturia inaequalis* were discharged naturally from mature perithecia in apple leaves that had been soaked in water for a few minutes and placed on wet filter paper in glass dishes inverted over dry cover glasses. Precautions were taken against wetting of spores and cover glasses.

The mean germination of the ascospores on dry glass was high at a rela-

tive humidity of 100 per cent, low at 99 per cent, and zero at 98.7 per cent (Fig. 3, A). The mean percentage germination at a relative humidity of 100 per cent was slightly lower than that of controls in redistilled water in which the germination after 3, 4, 7, 16, 24, and 48 hours, respectively, was 36, 66, 73, 97, 96, and 97 per cent. The mean germination at a relative humidity of 100 per cent after 24 and 48 hours, respectively, was 85 ± 4 and 94 ± 2 per cent. At relative humidities of 99.85, 99.6, and 99.4 per cent the percentages of germination were progressively lower than at 100 per cent. The mean germination at a relative humidity of 99.6 per cent after 24 hours in 5 series was 28.2 ± 5.4 per cent. At a relative humidity of 99 per cent the

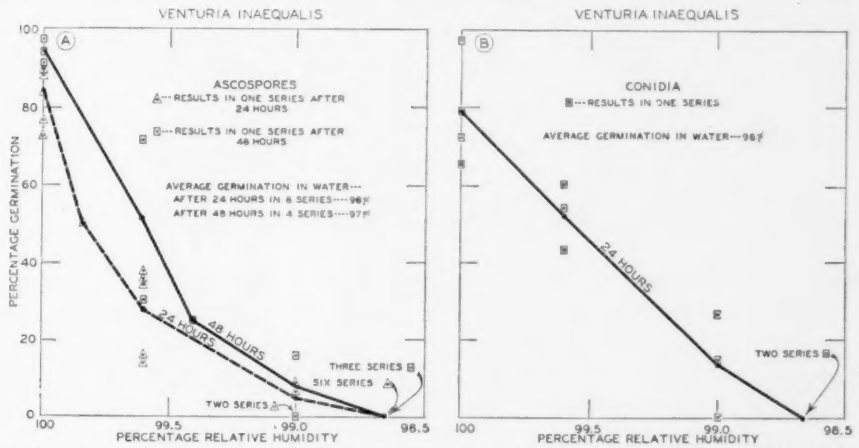

FIG. 3. Relation of relative humidity to germination of spores of *Venturia inaequalis* on glass at $20° \pm 0.02°$ C. A. Ascospores. B. Conidia.

mean germination in the various series ranged between 0 and 23 per cent, whereas no germination occurred at relative humidities of 98.7 per cent or below.

The mean length of 50 to 150 germ tubes from spores in water and at various relative humidities for 24 hours and 48 hours, respectively, are presented in figure 4. The relation of time to cumulative increase in length of germ tubes from spores in water also is shown. The mean length of the germ tubes was reduced with decreasing relative humidities. The mean length of the germ tubes at a relative humidity of 99.6 per cent was significantly less than at 100 per cent.

Comparative tests were made with ascospores of *Venturia inaequalis* on the surface of cover glasses, paraffin-coated cover glasses, and the ventral surface of a mature apple leaf. The experiments were made in an attempt to determine whether the chemical or physical properties of the spore-carriers might alter the relation of relative humidity to the germination of the spores. The number of spores counted on each surface at each relative humidity ranged between 300 and 1,700.

Results from the comparative use of different surfaces as spore-carriers

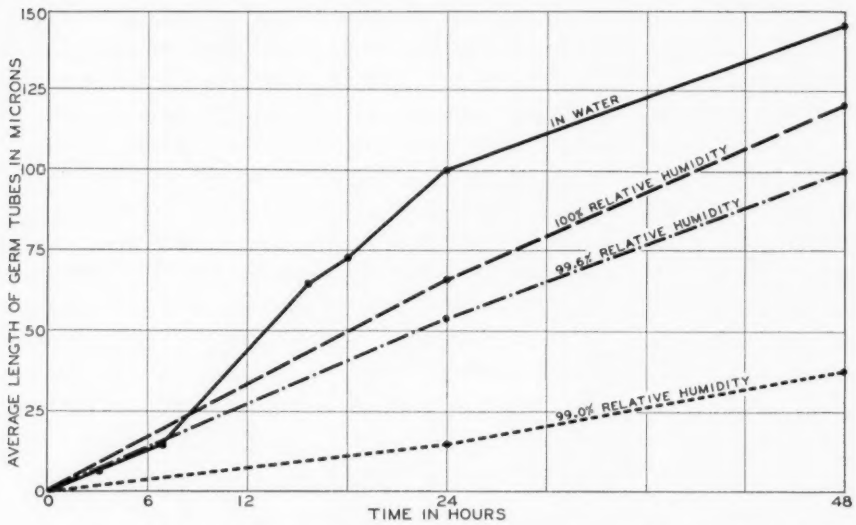

FIG. 4. Relation of water and relative humidity to length of germ tubes of ascospores of *Venturia inaequalis* on glass at 20° ± 0.02° C.

are shown in figure 5. The variations in the percentage germination on the three types of surfaces were relatively small. Germination did not occur at a relative humidity of 98.7 per cent on any of the surfaces. While these data are too limited to justify conclusions, they suggest that the different surfaces used did not exert any important influence on germination.

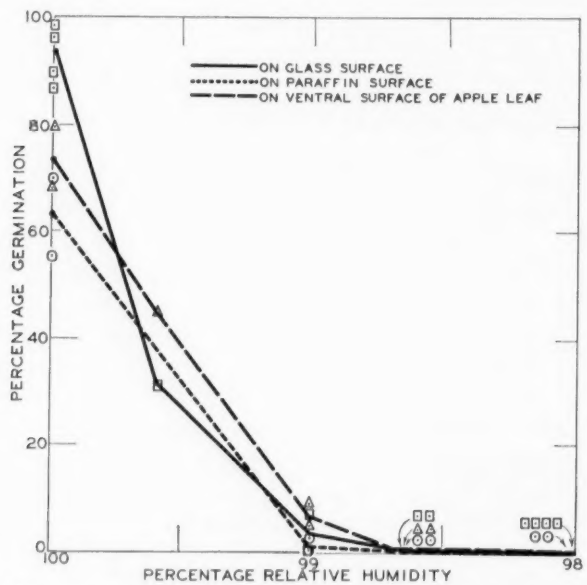

FIG. 5. Effect of various surfaces on the relation of relative humidity to germination of ascospores of *Venturia inequalis* at 20° ± 0.02° C.

Conidia of Venturia inaequalis

Conidia of *Venturia inaequalis* from scab lesions that had been macroscopically visible for 3 or 4 days were brushed lightly over dry cover glasses. The lesions had been produced on vigorous leaves of a potted Fameuse apple tree in the greenhouse. A monoascosporic culture was applied to the tree as inoculum. Two to 4 series were made at relative humidities from 98 to 100 per cent. The total number of spores counted at each relative humidity ranged between 1300 and 2700.

The results, graphically presented in figure 3, B, indicate that the conidia and ascospores of *Venturia inaequalis* have similar relative humidity requirements for germination. The mean germination of conidia in water and at relative humidities of 100, 99.6, 99.0, 98.7, and 98.0 per cent, respectively, was 96 ± 2, 79 ± 7, 52 ± 4, 14 ± 8, 0, and 0 per cent. The germination of the conidia was not quite so uniform as that of the ascospores. However, it is noteworthy that neither conidia nor ascospores on dry glass germinated at relative humidities of 98.7 per cent and below.

Conidia in redistilled water for 4 hours had germ tubes 10 to 15 μ in length. The germination of conidia after 5½ hours in water was 85 per cent, the average length of germ tubes being 20 μ. The mean length of 25 germ tubes from spores in water for 24 hours was 146 μ.

Two comparative series of experiments for 24 hours were made with *dry* conidia brushed from leaf lesions over cover glasses and with *wet* conidia obtained in suspension from leaf lesions and atomized over cover glasses. The water on the cover glasses with the *wet* conidia was dried in an air stream. The *wet* condia were in water for a total period of 30 to 40 minutes before they were dried. At relative humidities of 100, 99.6, 99.0, 98.0, and 92.0 per cent, respectively, the mean germination of the *wet* conidia was 74, 67, 9, 0, and 0 per cent, whereas that of the *dry* conidia, at the same relative humidities, was 79, 52, 14, 0, and 0 per cent. Evidently, the wetting of the conidia for 30 or 40 minutes did not appreciably affect either the minimum relative humidity at which germination would occur or the mean percentage germination at relative humidities from 99 to 100 per cent.

Urediospores of Puccinia coronata, P. graminis tritici, and P. graminis avenae

Spore material of *Puccinia coronata* collected in Wisconsin and of *P. graminis tritici* race 56 and *P. graminis avenae* race 2 received from E. C. Stakman served to establish these fungi on susceptible plants on which inoculum could be maintained. Subsequent inoculations were made on State's Pride (Wis. Ped. No. 7–7) oat seedlings or on Marquis spring-wheat seedlings growing in the greenhouse. Urediospores were shaken or brushed lightly over a watch glass from uredia that had been macroscopically visible for 3 to 7 days on vigorous leaves. The spores were mixed lightly with the camel-hair brush with which they were dusted over the glass or paraffin spore carriers. One to 4 series for 24 hours and 1 to 2 for 48 were made

with spores of each fungus at humidities ranging from 92 to 100 per cent. A total of 500 to 5,000 spores was counted for each relative humidity on each type of spore carrier.

The mean germination of spores of *Puccinia coronata* on or in thin drops of water on cover glasses was 83 per cent after 24 hours and 89 per cent after 48 hours. In one experiment the mean germination in water after 4½ hours was 95 per cent. The germination on glass at a relative humidity of 100 per cent was considerably lower than in water. At a relative humidity of 99 per cent the average germination on glass was 15 per cent after 24 hours and 32 per cent after 48 hours. None of the spores on paraffin for 48 hours and only 0.2 per cent of those on glass germinated at a relative humidity of 98 per cent. Spores on either glass or paraffin exposed to relative humidities of 95 or 92 per cent for 48 hours failed to germinate. The percentages of germination of spores on paraffin at relative humidities of 99 or 100 per cent were somewhat lower than those on glass.

The mean length of the germ tubes from spores of *Puccinia coronata* on or in a thin drop of redistilled water was 192 μ after 4½ hours, 273 μ after 5½ hours, and 468 μ after 9 hours. The average lengths of the germ tubes after 24 hours on glass at relative humidities of 100, 99, 98.7, and 98 per cent, respectively, were approximately 555, 289, 172, and 76 μ. The average length of the germ tubes after 48 hours was not appreciably greater than after 24 hours. The germ tubes at relative humidities of 100 per cent or below did not show nearly so much of the knotty or branching tendency as did the germ tubes of *P. graminis* at the same relative humidities.

Spores of *P. graminis tritici* placed on or in redistilled water for 6 hours exhibited from 95 to 100 per cent germination. The mean germination on or in water on glass after 24 and 48 hours, respectively, was 98 and 97 per cent and on paraffin after 24 hours was 99 per cent. Germination of spores in water started before 2 hours had elapsed. The mean germination on glass at a relative humidity of 100 per cent was 81 per cent after 24 hours and 86 per cent after 48. The percentage germination at a relative humidity of 99 per cent was much less than that at 100, whereas, at 98 per cent, the percentage germination was very small, being zero in some series. At a relative humidity of 95 per cent a few spores had very short germ tubes in one test on glass for 24 hours and in a test on paraffin for 48 hours. The percentage germination at a relative humidity of 100 per cent was slightly higher on paraffin than on glass, but the variations in percentages of germination between the two surfaces in different series were not always consistent. Therefore, it is believed that the differences in the percentages of germination between glass and paraffin at 100 per cent relative humidity were not significant. At relative humidities of 99 and 98 per cent in tests for 24 and 48 hours, the percentage germination on glass was slightly higher than on paraffin.

The mean length of germ tubes from spores in water after 2, 5, 6, 12, and 48 hours, respectively, was 80, 200, 247, 654, and 590 μ. The germ tubes of

spores on dry glass at relative humidities of 100 per cent or less were very knotty or branched, whereas, in water, the germ tubes were only slightly knotted. Length of germ tubes decreased in direct relation to decrease in relative humidity.

The results with spores of *Puccinia graminis avenae* were similar to those with *P. graminis tritici.* Spores on or in redistilled water for 9 hours produced germ tubes averaging 390 μ in length. Those in water on glass for 24 or 48 hours, respectively, showed 87 and 99 per cent germination. The percentage germination of spores on glass at a relative humidity of 100 per cent was somewhat lower than in redistilled water, and at 98 per cent was very low. Germination on paraffin was, at most relative humidities, somewhat lower than on glass. The mean lengths of the germ tubes from spores of *P. graminis avenae* after 24 hours at relative humidities of 99 and 98 per cent, respectively, were 150 and 58 μ.

A comparative series of tests with urediospores of *Puccinia coronata, P. graminis tritici,* and *P. graminis avenae* was made. A glass and a paraffin spore carrier for each of the 3 fungi were placed in each of 4 humidity chambers at each of 4 relative humidities; namely, 100, 99, 98, and 95 per cent. Two of the chambers at each relative humidity were used in a 24-hour series and the other two were allowed to continue in a 48-hour series. The number of spores counted on most of the spore carriers was 500, ranging between 300 and 1000. The results (Fig. 6) indicate that the germination of the urediospores of *P. coronata* is slightly lower than that of the two stem rust fungi. The urediospores of *P. coronata* did not germinate at relative humidities of 98 per cent or below, whereas a low percentage of the urediospores of *P. graminis tritici* and *P. graminis avenae* did germinate at a relative humidity of 98 per cent.

Chlamydospores of Ustilago hordei and U. nuda

The experiments with *Ustilago hordei* were made with chlamydospores that were brushed from a few smutted heads of barley into a vial where they were kept until used. In preliminary tests germination of the 6- to 8-month-old spores on water ranged between 30 and 57 per cent. Several series for 48 hours were made on glass and paraffin at 8 relative humidities between 81 and 100 per cent. A total of 1000 to 9000 spores was counted at each relative humidity.

The results of two representative series are presented graphically in figure 7, A. In the controls spores on redistilled water started to germinate within 3 hours and germination after 48 hours on redistilled water on glass or paraffin was approximately 40 per cent. The mean germination on glass at a relative humidity of 100 per cent was less than half that on redistilled water. At a relative humidity of 99 per cent the mean germination was only slightly lower than at 100 per cent. The mean percentages of germination on glass at relative humidities of 95, 98, 99, and 100 per cent, respec-

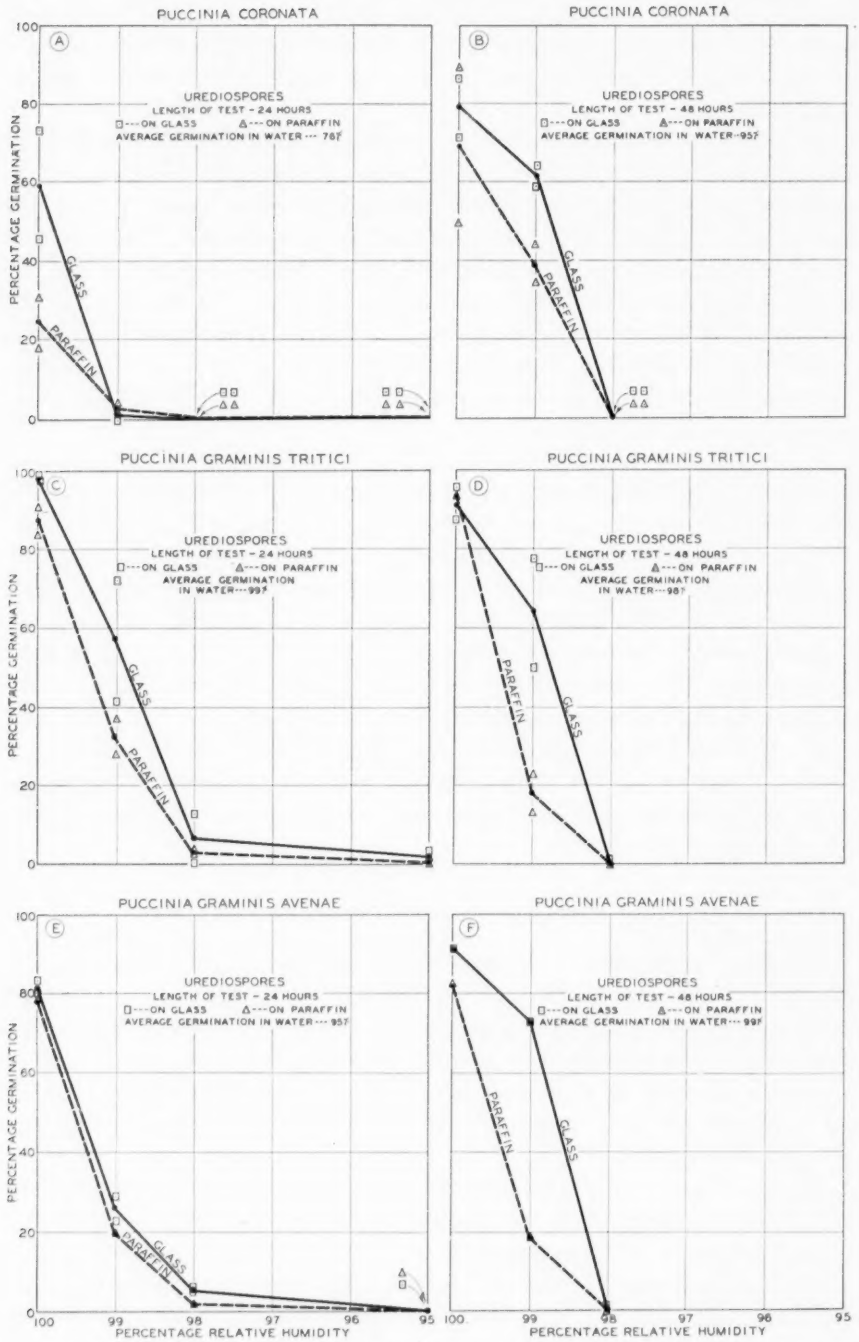

FIG. 6. Relation of relative humidity to germination of urediospores on glass and paraffin in a single series at 20° ± 0.02° C. A, B. *Puccinia coronata.* C, D. *P. graminis tritici* (race 56). E, F. *P. graminis avenae* (race 2).

tively, were almost always higher than on paraffin. Spores on glass or paraffin failed to germinate at a relative humidity of 93 per cent or below.

Production of sporidia was fairly low on redistilled water, very sparse at a relative humidity of 100 per cent, and absent at 99 per cent or below.

Long primary basidial hyphae grew from the basidia at relative humidities of 100, 99, 98, or 95 per cent. The average lengths of the basidia, together with the longest basidial hyphae at a relative humidity of 100 per cent after 24, 27, 35, and 48 hours, respectively, were 62, 81, 150 and 217 μ. The basidial hyphae at the lower relative humidities at which germina-

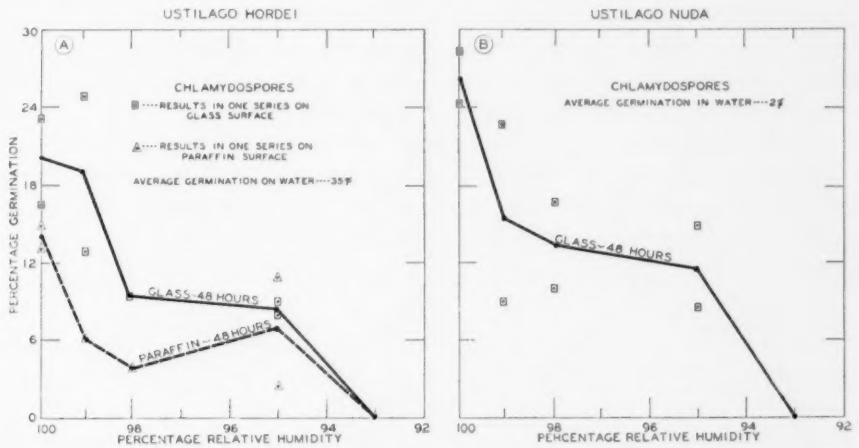

FIG. 7. Relation of relative humidity to germination of chlamydospores at 20° ± 0.02° C. A. *Ustilago hordei*. B. *U. nuda*.

tion occurred were only slightly shorter than those at the higher relative humidities.

The chlamydospores of *Ustilago nuda* were obtained from a few smutted heads of barley of Hybrid × 163. The germination of the chlamydospores in or on water in preliminary tests ranged between 1 and 13 per cent. Two series were made for 48 hours on glass at various relative humidities from 88 to 100 per cent. The total number of spores counted at each relative humidity ranged between 2,000 and 23,000.

The results of the experiments on spores of *Ustilago nuda* are given in figure 7, B. In the controls the average germination was 2 per cent in redistilled water and 7 per cent on the surface of it. On dry glass at relative humidities from 100 to 95 per cent the percentage germination was higher than on water. The percentage germination at a relative humidity of 100 per cent was significantly greater than at 98, whereas it was lower at 95 than at 99 per cent. At 93 per cent relative humidity or below no germination occurred in two series of experiments.

The germ tubes from spores in water were relatively short; whereas, at relative humidities of 100, 99, 98, or 95 per cent, they were very long. The

average length of 25 germ tubes from spores in water and at a relative humidity of 95 per cent, respectively, for 48 hours was 33 and 222 μ.

Conidia of Erysiphe polygoni from Red Clover, Cabbage, Evening Primrose and E. graminis hordei

Conidia of *Erysiphe polygoni* from young sporulating lesions on young, fully expanded leaves of red clover growing in the greenhouse were shaken over a watch glass. The spores were stirred lightly with the camel-hair brush with which the conidia were later dusted over clean, dry cover glasses. These spore carriers were promptly placed at various relative humidities from 0 to 100 per cent. It should be noted that the conidia on mildew lesions from which spores were to be obtained were brushed off 24 hours prior to an experimental series. Germination at the start of the tests was almost invariably zero and was never more than 1 per cent.

Experiments were made with conidia collected in the early afternoon on bright sunny days. The results from a representative series are presented graphically in figure 8, A. The mean length of 75 germ tubes of conidia of *Erysiphe polygoni* at each of the various relative humidities is shown in figure 8, B.

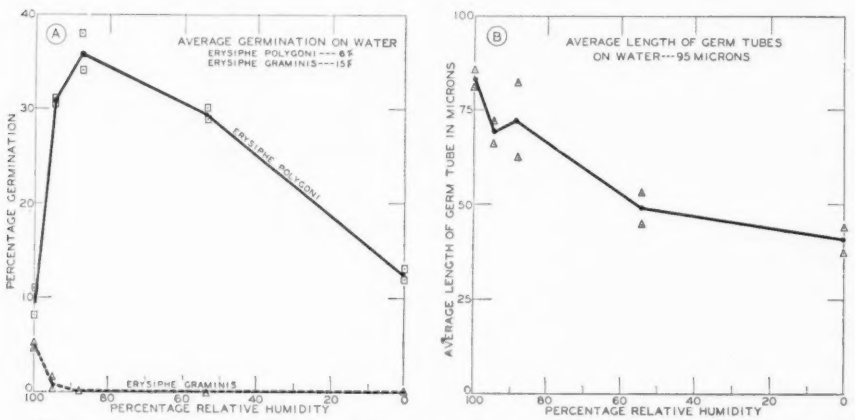

FIG. 8. Relation of relative humidity to germination of conidia of *Erysiphe polygoni* from red clover and of *E. graminis hordei* in a single series on glass at 20° ± 0.02° C. for 24 hours. A. Percentage germination. B. Average length of germ tubes of *E. polygoni*.

Germination of the spores on water was much lower than at high relative humidities. The percentage germination at relative humidities of 98, 95, or 88 per cent was usually higher than at 55 or 0 per cent.

The germ tubes from spores at most relative humidities were relatively short in comparison to the length of the conidia. The average length of 75 germ tubes from conidia after 24 hours at relative humidities of 0, 55, 88, 95, 96, and 100 per cent, and on water, respectively, was 37, 48, 75, 78, 81, 91, and 102 μ. It is evident, therefore, that germ-tube length was in direct relation to relative humidity.

The percentage of shriveled or dead spores at the beginning of the tests varied widely in different series. In one series, 21 per cent of the spores were shriveled at the start. At the end of the 24-hour series, the percentage of the spores shriveled at relative humidities of 0, 55, 88, 96, and 100 per cent, and on water, respectively, was 100, 82, 72, 63, 45, and 17. The vitality of many of the nonshriveled, apparently viable conidia was low, for they failed to germinate at the specific relative humidities.

Conidia submerged in water usually failed to germinate. The walls of many of them were ruptured, and the contents of most of the others had become granular or disorganized.

The spores, after dislodgment, are very short-lived under most conditions. The percentage germination in most series was less than 50 per cent at the most favorable relative humidity. In one series in which conidia were collected following 3 days of sunless cloudy weather, germination at all humidities from 0 to 100 was less than 10 per cent. The germinability of conidia in a sample seems influenced greatly by age of lesions and by temperature, moisture, and light conditions during the few days before sampling.

In two series of experiments with conidia of *Erysiphe polygoni* from cabbage, the relation of relative humidity to percentage germination proved similar to that of conidia of *E. polygoni* from clover. The average germination at relative humidities of 0, 20, 32, 55, 81, 95, and 100 ranged from 14 to 39 per cent. No consistent differences in the percentage germination were found at any of the relative humidities from 0 to 100 per cent, although there seemed to be a tendency for the percentage germination to be slightly higher at the higher relative humidities than over anhydrous $CaCl_2$. The germination in hanging drops of distilled water ranged from 1 to 6 per cent. Spores on dry glass slides exposed to the 35–50 per cent relative humidity in the laboratory for 22 hours showed 35 per cent germination.

In a 21-hour series the average germination of 500 to 1,000 conidia of *Erysiphe polygoni* from evening primrose at relative humidities of 100, 95, 88, 55, and 0 per cent, respectively, was 33, 24, 21, 5, and 9 per cent. At the start of the series, 51 per cent of the spores were shriveled and 1 per cent had germinated. Spores on dry glass slides, exposed to laboratory atmosphere for 21 hours, averaged 15 per cent germination.

Two series of experiments were made with conidia of *E. graminis hordei* from greenhouse-grown barley (C.I. 934) plants. The spores were shaken over a watch glass from leaves on which the mildew lesions had been macroscopically visible 3 days. The spores after being stirred on the watch glass were dusted over dry cover glasses. From 2,000 to 4,000 conidia were counted at each relative humidity.

The results from a representative series are shown in figure 8, A. The variation in results from the two series was relatively small. The average germination on water after 24 hours was 14.5 per cent, the germ tubes from some spores being 184 μ long. The mean length of 25 germ tubes from spores on water for 6, 25, and 54 hours, respectively, was 30, 139, and 130 μ.

The average germination at relative humidities of 100 and 95 per cent, respectively, was 5 and 0.8 per cent. The spores of this series showed almost no germination at relative humidities of 88 per cent or below. The length of the germ tubes at a relative humidity of 95 per cent was much less than at a relative humidity of 100 per cent.

DISCUSSION

The method applied in the spore-germination tests at high relative humidities, as herein recorded has the following advantages: (1) relative humidity and temperature are accurately controlled, (2) the variables to which spores are subjected are reduced to the relative humidity and the nature of the spore carriers, (3) a large number of tests can be made in a single series, and (4) the apparatus is relatively inexpensive.

Probably the most satisfactory and reliable method of controlling relative humidity for spore germination tests is obtained through aqueous solutions of pure nonvolatile chemicals inside a closed system maintained at an accurately controlled temperature. However, to prevent stratification of the solution or the atmosphere within the chamber or system and to increase the diffusion of water vapor throughout the chamber, it is desirable to use a small chamber and to agitate both solution and atmosphere by some such device as is described in the foregoing. The relative humidity in a closed system over a saturated salt solution or a chemical solution of a certain concentration at a stated temperature remains constant, provided the temperature does not vary and changes within the solution do not occur. However, with fluctuations in the temperature, equilibrium is disturbed and considerable changes in the relative humidity may occur before equilibrium is restored. Quick changes in temperature may produce abrupt changes in relative humidity, while the effect of a very small, gradual drift in temperature may be almost nullified by action of the control solution. The time necessary for the solution and atmosphere to reach equilibrium following changes in the temperature may be reduced by movement of the solution and atmosphere within the system. Fluctuations of temperature must be minimized if control of relative humidities close to 100 per cent is to be accurately maintained.

The slight temperature fluctuations of 0.02° C. within the water bath usually occurred gradually over a period of several hours. Therefore, the temperature changes at the surface of the spore carriers within the chamber must have been more gradual and of less magnitude than were the recorded temperature changes for the water bath. Since temperature changes within the chambers must have been extremely slight, the relative humidities must have been rigidly controlled.

Direct measurements of relative humidity were not made because of lack of a feasible method for making accurate determinations of high relative humidities within small closed chambers. However, spore germination served as a very delicate biological indicator of accuracy of control of the

high relative humidities employed. Conidia of *Sclerotinia fructicola,* for example, germinated freely in water but did not germinate at a relative humidity of 100 per cent; and ascospores of *Venturia inaequalis* showed significantly different percentages of germination and lengths of germ tube at each of the following relative humidities: 100, 99.85, 99.6, 99.0, and 98.7 per cent.

It is recognized that in the tests in which the theoretical relative humidity is herein reported as 100 per cent, condensation was not always avoided. When it could be observed, it was only in a very thin film of precipitated moisture that was evanescent when the 100 per cent chamber was opened. However, germination of spores at this humidity differed consistently from that on or in drops of water. No visible condensation on the spore-carrying surfaces occurred at relative humidities of 99.85 per cent or below.

The question as to whether the nature of the spore carriers might affect the relation of relative humidity to germination was investigated by using glass, paraffin, and quartz in comparative tests with spores of several fungi. Since these substances have different physical and chemical properties, the tendency for condensation of water vapor on one might be greater than on another. The average percentage germination of spores of 3 species of cereal rust fungi, of *Ustilago hordei,* and of *Venturia inaequalis* was lower in most tests on paraffin than on glass. Yet, no great differences in results on these spore carriers were evident, for the germination of conidia of *Sclerotinia fructicola* did not occur on glass, paraffin, or quartz at relative humidities of 100 per cent or below. Since the percentages of germination on glass were usually slightly higher and more uniform than on paraffin and because glass was more convenient to use than paraffin or quartz, clean dry glass was employed in the greater portion of the germination studies.

Spores of various phytopathogenic fungi on the nonnutritive substrata used appeared to vary greatly in their ability to germinate at different relative humidities or in the absence of liquid water. The conidia of *Sclerotinia fructicola* on a nonnutritive surface apparently required contact with liquid water before they were able to germinate. On the other hand, the germination of ascospores and conidia of *Venturia inaequalis* was very high at a relative humidity of 100 per cent, medium at 99.6 per cent, low at 99.0 per cent, and zero at 98.7 per cent. Prior to the tests reported herein, it had not been demonstrated that the spores of the apple scab fungus could germinate at high relative humidities in the absence of liquid water. The minimum relative humidity for germination of urediospores of *Puccinia coronata* appeared to be at or slightly above 98.0 per cent, whereas, for urediospores of *P. graminis tritici* (race 56) and *P. graminis avenae* (race 2), it apparently was at or slightly below 98.0 per cent. The germination of some chlamydospores of *Ustilago hordei* and *U. nuda* occurred at a relative humidity of 95 per cent, but not at 93 per cent. Conidia of *Erysiphe polygoni* from red clover, cabbage, and evening primrose on glass germinated at all the relative humidities tested from 100 per cent to approximately 0 per cent. Conidia

of *E. graminis hordei* appeared to be much more sensitive to moderate and low humidities than did the conidia of *E. polygoni.*

It is evident that spores of different fungi vary in their moisture requirements for germination. To explain these variations more knowledge concerning the fundamental mechanism, organization and nature of the membranes and protoplasts of a spore is needed. The membranes of different spores of the same species, as well as of different fungi, probably are extremely variable. The reserves in spores of different fungi vary greatly in composition and quantity and doubtless also, in their utilization of water. In rust spores, the reserves may be largely oils, whereas in conidia of the powdery-mildew fungi there may be stored materials of other types. Corner (7) suggested that the conidia of powdery-mildew fungi probably carry the water for germination within the large vacuoles in the spores. Brodie and Neufeld (3) stated that there is probably very little water present in the conidium of *Erysiphe polygoni,* the protoplast consisting of a gel-like material. Upon exposure of the papilla of the conidium to air, CO_2 would be released and respiration would begin. As a result, the viscid protoplast would be converted into materials more labile and voluminous, and the increase in volume necessary for the production of a germ tube might come from this source alone. The wettability of the spore wall and its permeability to water may be very important factors in determining the relation of relative humidity to germination.

Humidity is an important factor affecting incitation and development of plant diseases, especially those caused by pathogenic fungi. Epidemics of brown rot of fruits, caused by *Sclerotinia fructicola* and related species, follow periods of wet, rainy weather, since the spores require contact with water for germination. Apple scab (*Venturia inaequalis*) results from infections during or immediately following rain periods, free water, primarily rain, being essential to the discharge of ascospores and abscission and dissemination of conidia. However, at or near the optimum temperature, some of the spores can germinate at relative humidities of 99 per cent or above, and most of the spores germinate at a relative humidity of 100 per cent or in water. Many investigators have stated that water on cereal plants was required for their infection by urediospores of various species of *Puccinia.* Lauritzen (17) and others, however, have reported that the presence of water on the leaves was not necessary, because relative humidities approaching saturation were sufficient to permit infection. It was found in the present work that the fresh urediospores of *P. coronata, P. graminis tritici,* and *P. graminis avenae* on glass or paraffin germinated at relative humidities as low as 98 per cent in the absence of water, although contact with water appeared to be optimum for germination. Several workers have shown that heavier infection with some smut fungi occurs in medium dry than in wet soil. In the study reported here, it was demonstrated that chlamydospores of *Ustilago nuda* and *U. hordei* germinated at relative humidities of 95 to 100 per cent. Chlamydospores in water germinated

poorly, whereas, on the surface of water, germination was fair. Thus, the heavier smut infection at medium to low soil moistures may be influenced by the ability of the spores to germinate at relative humidities below saturation. Infection with spores of many powdery mildews is readily accomplished on leaves of plants that do not become wet with water. That is possible because the conidia are able to germinate at relative humidities considerably below 100 per cent.

SUMMARY

A method for accurately controlling high relative humidities for spore germination tests was developed. Spores were discharged naturally or dusted with camel-hair brush over clean dry spore carriers. The carriers were then suspended in sealed glass chambers over sucrose solutions, saturated salt solutions, or redistilled water, the liquids used to secure the desired relative humidities, which were computed theoretically. The humidity chambers were placed in a wire container and completely submerged in a water bath the temperature of which was thermostatically maintained at $20.0° \pm 0.02°$ C. Stratification of the atmospheres or the solutions within the chambers was minimized by mechanically rocking the container of the chambers. Visible condensation upon the spore-carriers was precluded except to a limited degree at a relative humidity of 100 per cent.

Conidia of *Sclerotinia fructicola* germinated well in redistilled water, but failed to germinate in tests for 72 hours on glass, paraffin, or quartz at relative humidities of 100 per cent or below.

It was demonstrated for the first time that ascospores and condia of *Venturia inaequalis* could germinate on dry glass at relative humidities of 99 to 100 per cent.

The mean percentage germination of urediospores of *Puccinia coronata*, of *P. graminis tritici* (race 56), and of *P. graminis avenae* (race 2) on glass was high in water, lower at a relative humidity of 100 per cent, considerably lower at 99.0, and practically zero at 98 per cent.

It was demonstrated for the first time that chlamydospores of *Ustilago hordei* and *U. nuda* on dry glass or paraffin could germinate at relative humidities of 95 to 100 per cent but not at 93 per cent or below. Long basidial hyphae of *U. hordei* were produced at the relative humidities of 100 to 95 per cent whereas sporidia were produced from basidia of spores germinating on water.

Conidia of *Erysiphe polygoni* from red clover, cabbage, and evening primrose on dry glass germinated at relative humidities from 0 to 100 per cent. However, the germ tubes were very short and survived for a very short period of time at low relative humidities.

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THE VALUE OF SPERGON AS A SEED PROTECTANT FOR CANNING PEAS[1]

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INTRODUCTION

The pea-canning industry in Minnesota has in the past 4 decades developed into a large and nationally important business, with a total production in 1941 of 27,470 tons. This investigation was undertaken to obtain information on the damage done to the Minnesota pea crop by root rotting fungi, and to determine the value of certain chemicals as seed protectants. Research initiated at the New York Agricultural Experiment Station (Geneva) indicated that certain synthetic organic compounds might have some value as seed protectants for canning crops.[2] From several synthetic compounds, a material was selected on the basis of laboratory and greenhouse assay as having fungicidal value, and was thought to show some promise as a seed protectant. This material, originally known as United States Rubber material #120, has recently been named "Spergon," and is a complex, synthetic organic chemical containing no copper, sulphur, mercury or zinc. It is a light greenish-yellow, free flowing powder, relatively insoluble in water, and contains a buffering agent to permit its use in alkaline soils.[3] In extensive laboratory and greenhouse experiments it has been found highly fungicidal,[4] and, in field experiments with 25 varieties of vegetables and ornamental plants, has caused no seed injury except on peppers, egg plants, and garden beets, even when applied in excess.

METHODS

In the spring of 1940, cooperative seed-treatment experiments were established with canneries in 7 localities in southern Minnesota. Samples of Spergon, sufficient to treat enough seed for planting 1 acre, were mailed to each cooperator, who then treated his seed with this material at the rate of 2 ounces to the bushel. The treated seeds were then planted in 1-acre plots in the same field and contiguous to non-treated or other comparable plots. Attempts were made to maintain a uniform seeding rate in each section of the experimental field, although different rates of seeding were used by the different cooperators. The rates of seeding at the different localities ranged from 4 to $4\frac{1}{2}$ bushels of seed per acre. The investigation was continued in 1941, but at this time the experiments were confined to 1 cooperator, and

[1] Investigation made under the terms of a research fellowship sponsored by the United States Rubber Company, Passaic, New Jersey. Paper No. 1973, Scientific Journal Series of the Minnesota Agricultural Experiment Station.

[2] Cunningham, H. S., and E. G. Sharvelle. Organic seed protectants for lima beans. Phytopath. 30: 4. 1940.

[3] Spergon chemically is "Tetrachloro-para-benzoquinone."

[4] Felix, E. L. Tetrachloro para benzoquinone, an effective organic seed protectant. (Abstract) Phytopath. 32: 6. 1942.

plots of 10 acres for each treatment were established in a very large field of exceptionally uniform topography and soil type. Each plot was visited by one of the investigators at regular intervals, after planting, to obtain emergence data. Final stand counts, root-rot records, and yield data were obtained at harvest time.

Four criteria (stand of plants, disease rating, height of plants, and yields) were used at each location for comparing the Spergon-treated plots with the non-treated or other comparable plots.

a. *Stand of Plants* was determined by counting the number of plants within a measured area of 1 sq. yd., 10 such areas being selected at random throughout the plot. The average of these counts was used as the basis of comparison for the stand of plants in the different plots, the stand being expressed as the average number of plants per sq. yd.

b. *Disease Rating* was obtained by digging 50 plants at random from each plot. The entire sample was then examined immediately and the individual plants placed in 1 of 5 arbitrary classes, according to the severity of disease, as follows: 0, no infection; 1, slight discoloration of the stem; 2, discolored area diffused with slight necrosis; 3, marked discoloration, with well-defined necrosis; 4, stem or root completely girdled. The average of these 50 determinations was then used as the criterion for the severity of root rot in each plot.

c. *Height of Plants.* The 50 plants from each plot used in the previous determination of disease severity were measured, the length of roots and length of vine from crown to tip being recorded separately, the average of these determinations serving as the basis of comparison for the height of plants in each plot.

d. *Yield.* Each plot was accurately measured with a land wheel, the cutting of each plot was supervised by one of the writers and the yields were obtained directly from the viner in each location. Yields were finally computed on the basis of pounds of green shelled peas produced from a measured acre.

In addition to the above criteria grade information for the peas from each plot was obtained.

RESULTS

The results of the 1940 experiments are given in table 1 and are illustrated graphically in figures 1–4. From these data it can be seen that the stand was increased by seed treatment in every case. The percentage increases for the Spergon plots over the untreated plot being 23, 42, 41, 22, 11, and 1.2 per cent, an average of 23 per cent increase in stand. The increase obtained by the use of New Improved Ceresan also amounted to 23 per cent, while nitrogen inoculation increased the stand by 18.5 and 1.6 per cent, the average being 10 per cent. It was possible at all of the locations to pick out the nontreated plot by the uneven stand and the general prevalence of bare areas, usually scattered uniformly throughout the plot.

TABLE 1.—*Summary of field experiments with Spergon on pea seeds in Minnesota, 1940*

Location	Variety	Treatment	Date of planting	Date of harvest	Av. stand[a]	Av. length of plants (in.)[b]			Infection rating[c]	Yield per acre
						Crown–tip	Roots	Total		
										lb.
Plainview (Schultz)	Prince of Wales	Check	May 17		65.3	17.5	3.5	21.0	2.0	
		Spergon	"		111.8	22.8	4.2	27.5	0.7	
Plainview (Seefeldt)	Prince of Wales	Check	May 18		56.6	21.0	3.6	24.6	2.2	
		Spergon	"		97.3	20.10	3.3	23.4	1.0	
Blue Earth	Alaskan	Check	April 24	June 25	96.0	21.7	5.1	26.8	3.5	1960.0
		Spergon	"	"	106.7	24.4	4.7	29.1	1.9	2347.4
Spring Valley	Perfection	Check	May 2	July 9	79.0	17.5	2.1	19.6	2.6	2013.4
		N_2 inoc.	"	"	93.6	17.4	2.4	19.6	2.5	1221.9
		Spergon	"	"	111.1	17.2	3.0	20.7	1.8	2316.7
Dodge Center	Alaskan	Check			122.0	35.3	5.1	40.4	2.6	
		N_2 inoc.			124.0	35.0	4.7	39.7	2.8	
		Spergon and N_2 inoc.			120.0	39.0	5.2	44.3	2.1	
Fairmont	Teton	Check	May 4	July 3	66.8	30.4	4.2	34.6	3.4	1930.9
		N. Imp Ceresan	"	"	82.4	32.5	3.8	36.3	1.8	2268.7
		Spergon	"	"	84.6	33.3	4.7	38.0	2.5	2730.0
Owatonna	Alaskan	Check	May 2	July 2	103.0	25.7	4.7	30.4	2.8	2303.8
		Spergon	"	"	126.7	27.7	4.1	31.8	1.6	2243.4

[a] Stand: Each figure represents the average of ten counts made at random throughout the field 2 weeks before harvesting.
[b] Length: Figures represent the average of 50 plants.
[c] Infection rating: Refers to the degree of root rot present on the plants. Arbitrary infection classes of 0, 1, 2, 3, 4, were established. 0 represents no infection; 4, severe infection resulting in death of the plants. Figures represent the averages for 50 plants.

In every locality where Spergon was used the stand was very uniform over the entire field.

There was apparently no significant difference in the height of plants from treated and nontreated seed, although in every case but one, the plants

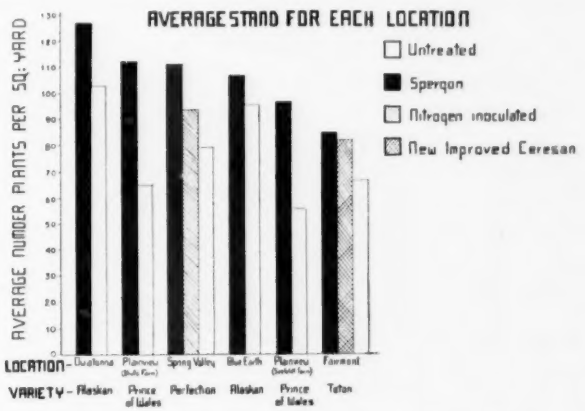

FIG. 1. Effect of seed treatment on the stand of peas.

from the former were slightly taller than those from nontreated seed. It was noticeable at all of the locations that the use of Spergon appeared to invigorate slightly the resultant plants, for, in addition to a perceptible increase in the height of plants, there was, apparently, also a more vigorous and extensive development of the root system. The above results were not analyzed for their statistical significance.

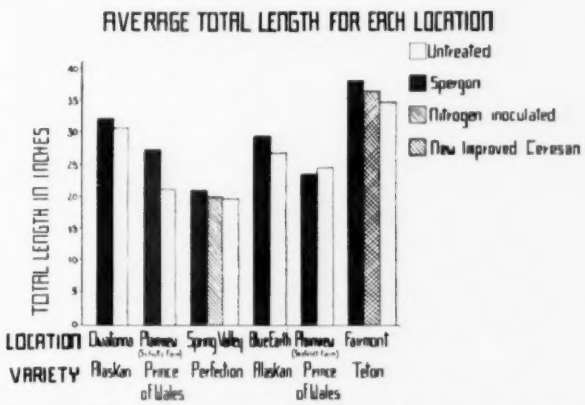

FIG. 2. Effect of seed treatment on the height of plants.

Using the scheme previously outlined, the severity of root-rot infection on the plants was determined in each plot at the different locations. The results given in table 1 are the averages of 50 determinations for each treatment, and from these results it is evident that seed treatment reduced the amount of root-rot development in every case. The percentage reduction in severity of root rot obtained by the use of Spergon, as compared with

the untreated plots was respectively 43, 65, 31, 46, 55, and 26 per cent, the average being 44 per cent. It would seem to be significant that seed treatment in the case of the variety Alaskan, which has been bred for

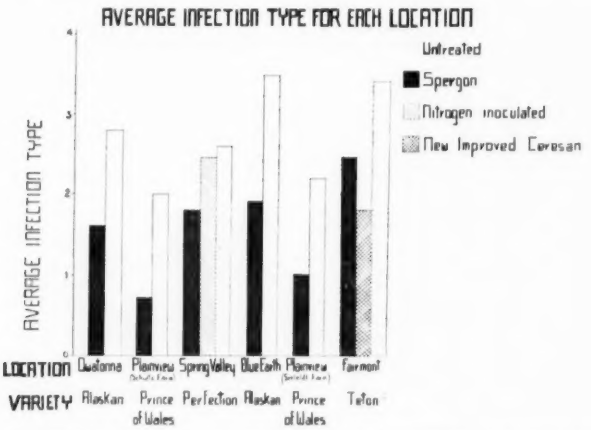

FIG. 3. Effect of seed treatment on the development of root rots.

resistance to Rhizoctonia root rot, resulted in a reduction of 43 and 46 per cent in the amount of root rot present. New Improved Ceresan in the one location in which it was used reduced the amount of root rot by 47 per cent.

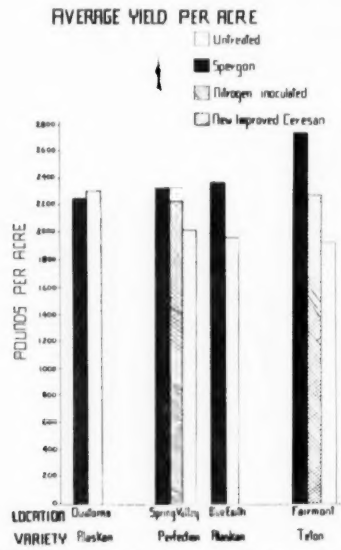

FIG. 4. Effect of seed treatment on the yield of peas.

The reduction in severity of root-rot infection obtained by the use of seed treatment is illustrated in figure 3.

The increases in yield obtained by the use of Spergon seed treatment, illustrated in figure 4, were, respectively, 303, 387, and 799 lb., while at

Owatonna a reduction of 60 lb. per acre was obtained. These figures in terms of percentage increase in yield as compared with the untreated plot may be expressed as an increase of 15, 20, and 41 per cent, with a reduction at Owatonna amounting to 2.16 per cent. Thus the average yield increase obtained by the use of Spergon amounted to 357 lb. of green, shelled peas to the acre, which is equivalent to an increase in production of 18 per cent over the untreated plot. New Improved Ceresan gave an increase of 338 lb. (17 per cent) as compared with 799 lb. (41 per cent) of green peas to the acre, obtained with Spergon at the same location.

Attempts also were made to determine whether seed treatment affected the quality of the crop grown from treated seed. In Minnesota the practice most commonly employed in the grading of canning peas is the use of the sample grader. In the grading process, employed at the canning factory, representative samples of the crop are passed over standard screens and the percentage of peas falling in the various sieve sizes determined, from which the field grade is computed by estimating the percentage weights of the peas falling in the desired sieve sizes. The number of sieve sizes used in determining the field grade varies with the variety of pea being tested; *i.e.*, in general the percentage weight of the 1, 2, and 3 sieve sizes would determine the field grade of the variety Alaskan, and the weight of sieve sizes 2, 3, 4, 5, and 6 would be used in determining the field grade of the large sweet varieties of peas. Thus, every packer is primarily interested in obtaining the greatest percentage possible of the desirable sieve sizes. In addition to the above method, a device known as the "tenderometer" is used by some packing plants to determine the quality of peas. This device measures the maximum force necessary to crush the sample. The reading obtained is an indication of the tenderness of the sample, that with the most desirable quality, having the lower reading. In this investigation, wherever possible, records were obtained on the quality of peas harvested from the various treated and nontreated plots. The records were obtained from the plant of the company concerned and the results are given in table 2. From

TABLE 2.—*Effect of seed treatment on pea quality as determined by sample grading and by tenderometer measurements*

Location	Variety	Treatment	Percentage of crop falling in each sieve							Tenderometer reading
			1	2	3	4	5	6	7 & 8	
Blue Earth	Alaskan	Nontreated	2	8	13	68	14	....	...	121
		Spergon	2	9	21	53	13	....	...	116
Fairmount	Teton	Nontreated	7	10	37	6	.....	31	9	.....
		N. Imp. Cer.	7	9	39	5	.....	33	9	.....
		Spergon	8	8	41	6	....	29	8	.....

these data it is apparent that seed treatment did not significantly affect the grade, although in the case of Alaskans a slight improvement is to be noted.

TABLE 3.—*Summary of field experiments with Spergon on Perfection peas at Spring Valley, Minnesota, 1941*

Treatment	Planting date	Date of harvest	Average length of plants in inches, June 5 and 6		Average length of plants in inches, June 25 and 26		Stand, June 5 and 6	Stand, June 25 and 26	Yield per acre in pounds
			Roots	Total	Roots	Total			
No treatment	April 30	July 6	3.3	14.3	6.8	27.4	91	86	2560.4
Spergon	May 2	July 8	3.9	17.3	7.3	28.7	107	87	2915.2
Spergon plus inoculation	May 1	July 7	3.2	15.5	7.2	31.5	105	93	2650.8

TABLE 4.—*Significance*[a] *of data on stand and plant length given in table 3*

Date taken	Comparison	Root length	Total vine length	Stand
June 5 and 6	Spergon—control	Highly significant[b]	Highly significant	Significant[c]
June 5 and 6	Spergon plus inoculation—control	Not significant	Significant	Significant
June 5 and 6	Spergon plus inoculation—Spergon	Highly significant	Highly significant	Not significant
June 25 and 26	Spergon—control	Not significant	Significant	Not significant
June 25 and 26	Spergon plus inoculation—control	Not significant	Highly significant	Not significant
June 25 and 26	Spergon plus inoculation—Spergon	Not significant	Highly significant	Not significant

[a] Significance was determined by comparing the means and using the standard error of the difference.
[b] Significance at 1 per cent level.
[c] Significance at 5 per cent level.

With the same variety, also, tenderometer readings indicated that the peas from Spergon-treated seed were of slightly better quality. The results, however, are not significant and it was concluded that seed treatment in these experiments neither increased nor lowered the quality of the crop.

In 1941 the size of experimental plots was increased to 10 acres, each, and all of the work was done in cooperation with the Reid Murdoch Canning Company on the Pattridge Farm at Spring Valley, Minnesota. All of the plots were planted to the variety Perfection at a seeding rate of 4.25 bu. per acre, and the seed was treated with Spergon applied at the rate of 2 oz. to the bushel. The results obtained in 1941 are given in table 3 and the statistical significance of these data is given in table 4.

From the records taken on June 5 and 6, one month after planting, and one month before harvest, it is apparent that a significant increase in stand resulted from the use of Spergon. The differences in root length and total vine length also were highly significant. A further significant increase in stand and length of vine was obtained when nitrogen inoculation was combined with the seed treatment. On the other hand, when similar records were taken on June 25 and 26, approximately 8 weeks after planting and 2 weeks before harvest, there was no significant difference in stand obtained by seed treatment. The differences in root length were not significant, but a significant difference in total vine length was again obtained, the difference being greater when nitrogen inoculation was combined with Spergon treatment.

An increase in yield of 13.9 per cent was obtained over nontreated seed by the use of Spergon, but no increase in yield was obtained when this treatment was combined with nitrogen inoculation. This failure of nitrogen inoculation plus Spergon to result in an increased yield is difficult to explain, although a marked decrease in yield as compared with nontreated seed resulted during the previous season, where nitrogen inoculation was used. Observations at that time indicated that the bacterial gel used for nitrogen-inoculation purposes may serve as a pabulum for root rotting fungi, increasing the injury that they cause. The results obtained in 1941 would indicate that seed treatment with Spergon is effective in combating pre-emergence damping off, but was not so effective in preventing root rot or post-emergence damping-off. The initial stimulus apparently was sufficient to result in an increase in yield. The same situation did not exist during the previous season as marked increases in stand were obtained at harvest time. Environmental conditions, however, were very different, 1940 being relatively dry until harvest time, whereas, in 1941, very wet conditions prevailed for several weeks before and until harvest time.

CONCLUSIONS

The increases in yield obtained by seed treatment in itself would seem to indicate that root rots of peas constitute a hazard to the pea industry in Minnesota, and during the course of this investigation, at least, must have

considerably reduced the yields. The prevalence of root rots is not generally recognized by the canners, and the losses resulting from such diseases are not at present realized, possibly because there has been no adequate standard of comparison, which experimental seed treatment experiments furnish, from which such losses could be computed. Isolations made from root-rot-infected plants in 1940 indicated that 3 fungi were involved as the cause of pea root rots in Minnesota. *Rhizoctonia* sp., *Fusarium martii* var. *pisi*, and *Aphanomyces euteiches* were isolated from diseased peas from different locations during 1940. In 1941 a species of *Pythium* was isolated from the diseased plants. The results obtained in this investigation indicate that Spergon was the most satisfactory seed protectant tested for canning peas in Minnesota, although it was not so effective in preventing the development of root rots late in the season, which also is true of other proprietary seed protectants. The apparent stimulation to seed treated with this material, as expressed by increased rate of emergence, greater total vine length and more vigorous root development, would seem to be sufficient to be responsible for increased yields when conditions suitable for the development of root rots prevail.

SUMMARY

Root rots of peas were present in epidemic proportions in the locations in which the above investigations were conducted during 1940 and 1941.

Seed treatment with Spergon in 1940 resulted in an average increase in stand of plants amounting to 23 per cent, New Improved Ceresan in one case gave the same increase, while nitrogen inoculation gave an increase in stand of 10 per cent over the untreated plot.

The height of plants was slightly increased in all cases but one by seed treatment in 1940, but the increases did not appear to be significant. The increases in total vine length obtained in 1941 were statistically significant. Spergon appeared to materially stimulate the root development of plants from seeds treated with this material.

The incidence of root rot was greatly reduced by seed treatment.

Increases in yield from 300 to 800 pounds of green, shelled peas to the acre were obtained at three locations by treating seed with Spergon, the average increase amounting to 357 pounds or 18 per cent over untreated seed in 1940.

Results obtained in 1941 substantiated the findings of 1940.

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THE ADHERENCE OF FIXED COPPER FUNGICIDES AS INFLUENCED BY SPRAY SUPPLEMENTS

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Supplementary materials are frequently added to spray mixtures of the fixed copper fungicides to improve such physical performance of the spray as coverage and adherence. The choice of materials used as spreaders and stickers has, in practice, been made largely by trial and error, and very little fundamental research has been reported on the performance of these substances. The purpose of this investigation was to study the effect of the commonly available supplementary materials upon the properties of copper-fungicide spray deposits in order to facilitate the selection of the most suitable materials.

MATERIALS

In order that the fungicidal efficiency of the spray be maintained, it is desirable that the chemical properties of the fungicide be not adversely affected by the addition of the supplementary materials. In other words, the supplements should be compatible with the fungicide.

A large number of supplements are being used commercially to adjust the physical properties of copper fungicide sprays (3). These materials may be classified according to their function as follows:

(a) Materials that act physically to spread the spray liquid, *e.g.*, sulphonated oils and sulphite waste liquor products.

(b) Materials that act physically to stick the spray deposit, *e.g.*, resins and mineral oils.

(c) Materials that act physically as spreaders and form gelatinous precipitates, which function as stickers, *e.g.*, soya flour, skim milk, etc.

During the last few years, materials of vegetable and animal origin, such as flours and casein materials, have been extensively used as spray adjuvants by experiment station research workers, as well as by commercial growers. These supplementary materials have been of particular value in spray mixtures, since they tend to impart a gelatinous character to the spray residue of fixed copper fungicide, and to improve its adherence (2). For this reason, materials possessing these properties were selected for this study. It will be noted from the analysis of these materials that they contain various amounts of proteins:

Composition of supplements included in adherence tests

Wheat flour—patent flour milled from southern wheat
(Analysis by General Mills laboratory)

	Per cent
Protein	8.600
Ash	.765
Moisture	13.5
Fat	.4

High protein wheat flour	
Protein	17.5
Soya flour—(Analysis by Central Soya Co. laboratory)	
Protein	53.5
Carbohydrates	30.0
Starch	Nil
Fat	.3
Ash	5.7
Moisture	7.0
Calcium caseinate	
Protein	42.0
(Calcium caseinate is a reaction product of casein and hydrated lime, which contains an excess of free lime.)	
Skim milk	
Protein	36.0

Since the basic sulphates of copper are most widely used for fungicidal purposes, a basic copper sulphate containing 26 per cent copper as metallic was selected for these studies.

LABORATORY TESTS

In order to investigate more completely the effect of supplements on copper-fungicide sprays, studies were conducted on the adherence of the spray deposit, the solubility of the copper fungicide in the spray mixture and the effect of soluble copper on the performance of the spray supplement.

Adherence Tests

The method of determining the adherence of the copper-fungicide spray deposit was patterned after that of Young and Beckenbach (6). The equipment used consisted of a DeVilbiss atomizer mounted in a permanent position over a container equipped with an agitator. The glass slides ($10'' \times 10''$) were supported at an angle of 45° at a distance of 30″ from the atomizer tip. A removable baffle imposed between the atomizer and the plate permitted deposition of the spray for definite intervals, and a uniform amount of material was deposited on replicate plates. The interval of deposition for each spray mixture was adjusted to the point of incipient running of the spray deposit in order to determine the maximum deposit obtainable. Quadruplicate slides were prepared for each spray mixture. The deposit on 2 plates was removed by washing with dilute sulphuric acid and analyzed for copper. The 2 remaining slides were dried at room temperature for 24 hours and then subjected to washing by artificial rain for 30 minutes. The volume of water running off from each plate was approximately 1000 ml. The residue was removed from the washed plates and analyzed for copper, as above.

Solubility Tests

The following method was used for determining the amount of soluble copper. A suspension of the copper fungicide and supplement was agitated for 16 hours at 76° F. in 3000 ml. of water, and allowed to settle for 24 hours. An aliquot portion of the clear supernatant liquid was withdrawn and analyzed for soluble copper.

Sorption Tests

The sorption of soluble copper by the supplements was determined by treating a suspension of the supplement with a known quantity of soluble copper at a definite concentration, allowing to stand for 24 hours, and analyzing an aliquot portion of the clear supernatant liquid for soluble copper. The difference between the initial and final copper content in solution represented the quantity of copper sorbed by the supplement.

The laboratory adherence tests were conducted with mixtures the composition of which were similar to those used in the field, *i.e.*, they contained the copper fungicide, supplement, and hydrated lime. However, in the solubility and sorption studies, lime was omitted from the systems in order to determine the effect of the supplements on the solubility of the copper fungicide and sorption of soluble copper.

LABORATORY RESULTS AND OBSERVATIONS

Adherence Studies

The results secured from the laboratory adherence tests of sprays containing the various supplements are summarized in table 1, and shown graphically in figure 1. The greatest average initial deposit of copper at

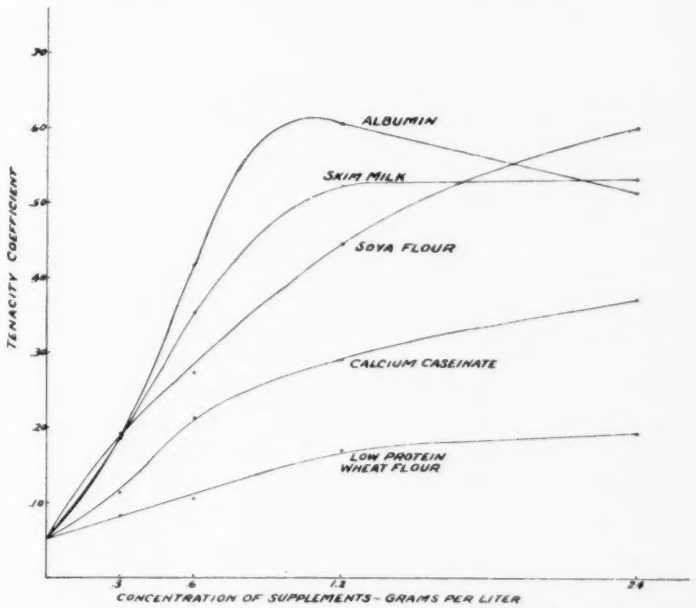

FIG. 1. Effect of spray supplements on the adherence of basic copper sulphate, laboratory tests.

the point of incipient running of the spray deposit was obtained with soya flour followed in decreasing order by wheat flour, calcium caseinate, skim milk, and albumin. The greatest average copper residue, after subjecting the dried deposits to artificial rainfall, was obtained with soya flour, fol-

TABLE 1.—*Effect of spray supplements on the adherence of basic copper sulphate. Summary of the results expressed as the average of the laboratory and 1939 and 1940 field spray tests*

Supplement[b]	Laboratory			Orchard 1939			Orchard 1940		
	Spray deposit Milligrams copper per 100 sq. in.		Tenacity coefficient[a]	Spray deposit Milligrams copper per 100 sq. in.		Tenacity coefficient	Spray deposit Milligrams copper per 100 sq. in.		Tenacity coefficient
	Initial	Residual		Initial (Cumulative)	Residual		Initial (Cumulative)	Residual	
Low-oil soya flour	13.25	5.32	.401	10.21	6.82	.668	20.89	14.58	.697
Low-protein wheat flour	11.30	1.54	.136	8.67	4.75	.548	11.34	6.91	.609
Skim milk	10.37	4.25	.410	10.39	8.52	.820	12.84	7.58	.590
Calcium caseinate	10.76	2.69	.250	7.91	4.35	.550	14.39	8.43	.586
Albumin	7.52	3.37	.448	9.81	6.19	.631	10.00	5.64	.564
None	7.97	.49	.051	6.66	3.13	.470	11.04	6.20	.561

[a] Tenacity coefficient = Ratio $\frac{\text{Residual spray deposit}}{\text{Initial spray deposit}}$.

[b] Dolomitic lime included in all sprays. Note:—Rainfall 1939 14.60 inches.
" 1940 16.10 "

Spray formula for 1939 and 1940 Orchard tests:—2.5 lb. Basic copper sulphate; 2.5 lb. lime; 100 gal. water.

The difference between the tenacity values of the laboratory and the field tests is undoubtedly due to the difference in the surface of the glass plates and of the apple foliage. The character of the surface has as much influence on adherence as the spray material itself.

lowed by skim milk, albumin, calcium caseinate, and wheat flour. From these data, the tenacity coefficient of the spray deposit (5) was calculated. This coefficient expresses the ratio of the residual deposit to the initial spray deposit. The sprays containing albumin as the supplement had the highest

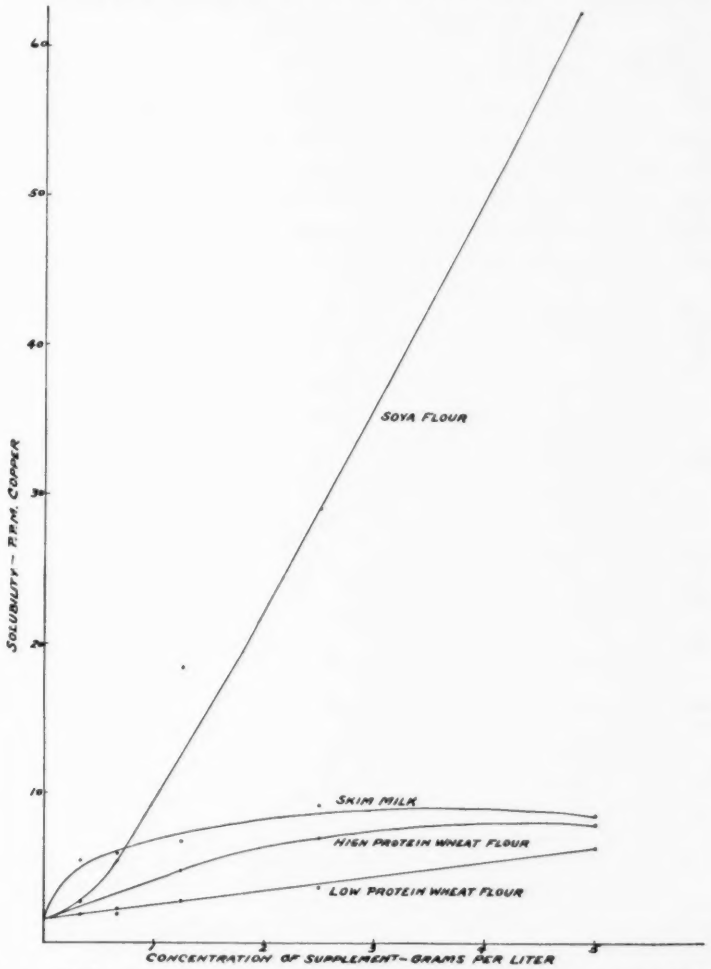

FIG. 2. Effect of spray supplements on the solubility of basic copper sulphate, laboratory tests.

average coefficient of tenacity, followed in decreasing order by those containing skim milk, soya flour, calcium caseinate, and wheat flour.

It is of interest to note that, although the sprays containing albumin had the highest coefficient of tenacity, the residual deposit for the albumin mixture was 37 per cent less than that for soya flour. The increased residual deposit which may be secured with supplementary materials is particularly important from the standpoint of the sufficiency of the coverage. Since the magnitude of the residue is a function of the initial spray deposit, the sup-

plementary materials which can be used to build up the initial deposit of copper are of particular value. From the above it is obvious that effective supplements, such as materials containing protein, may be used advantageously to build up the spray residue.

Solubility Studies

The loss of copper during rainfall may result from leaching of soluble copper, as well as from mechanical washing away of the solids from the spray residue. Although a primary object of using spray supplements is to secure improved adherence of the spray residue, the solubility of the copper fungicide should not be reduced by the supplements. It is apparent that the dissolving action of the spray supplement upon the fixed copper fungicide plays a very important role in the effectiveness of the spray, since

TABLE 2.—*Effect of spray supplements on the solubility of basic copper sulphate, and the retention of soluble copper by supplements. Laboratory tests*

Supplements	Soluble copper in copper fungicide supplement suspensions p.p.m. copper	Soluble copper sorbed by supplements mg. Cu/gm supplement	Analysis of sorption complex[a]		Adherence of sorption complex		
			Copper content	Water-soluble copper of total copper	Initial deposit mg. Cu per 100 sq. in.	Residual deposit mg. Cu per 100 sq. in.	Tenacity coefficient
		Per cent	*Per cent*				
Calcium caseinate	103.6	1300	2.62	2.0	.....	...	.....
Low-oil soya flour	24.5	2050	1.76	14.3	2.4	.6	.250
Powdered skim milk	7.2	762	1.86	25.3	.....	...	.....
High-protein wheat flour	5.4	810	0.58	27.6	.....	...	.....
Low-protein wheat flour	3.4	190	0.11	36.4	.88	.2	.227
None (check)	1.5	.....	.....	.....	.....	...	.....

[a] The term "Sorption Complex" represents the supplement containing sorbed copper.
Column 1—Determined by analysis from the suspension of basic copper sulphate and supplement in water.
Column 2—Represents the difference between the amount of copper in initial solution and final solution.
Column 3—Refers to the washed and dried residue from treatment of supplement with soluble copper.
Column 4—Determined from the suspension of residue (Column 3) in water.
The difference between the amount of soluble copper sorbed by the supplements (Column 2) and the copper content of the sorption complex (Column 3) is due to the fact that the supplements themselves are partially soluble in water.

it causes an increase in the concentration of available copper ion. The effect of this dissolving action upon the retention of copper is of particular interest. In this connection, a study was made on the effect of spray supplements upon the release of soluble copper from the copper fungicide.

The data from the solubility tests are shown graphically in figure 2, and are summarized in table 2. It was found that the highest concentration of

soluble copper was obtained in sprays containing calcium caseinate as the supplement (103.6 p.p.m. soluble copper) followed in decreasing order by soya flour (24.5 p.p.m.), powdered skim milk (7.2 p.p.m.), high-protein wheat flour (5.4 p.p.m.), low-protein wheat flour (3.4 p.p.m.) and the check, which contained no supplement (1.5 p.p.m.).

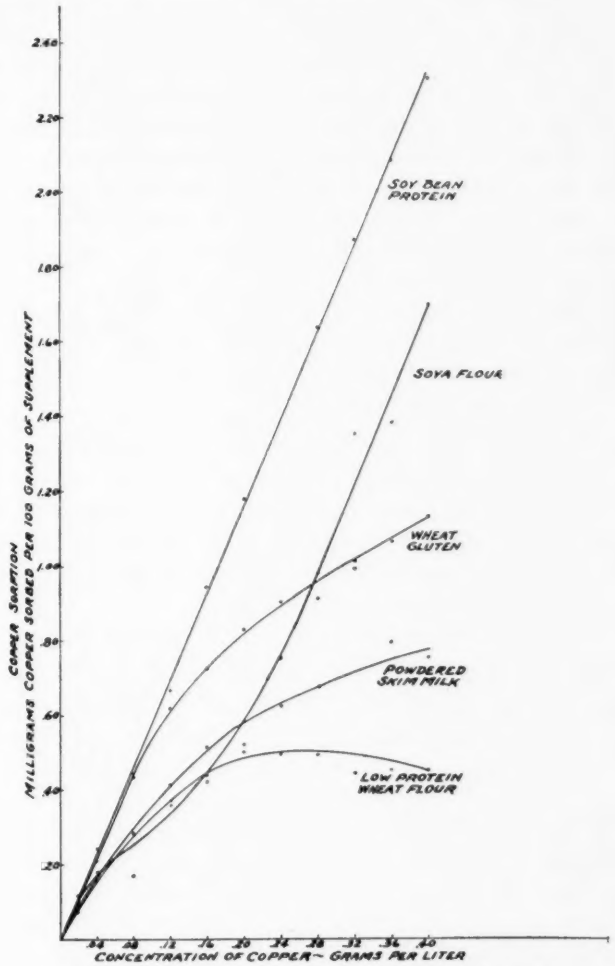

FIG. 3. Sorption of copper by spray supplements.

It was noted that soya flour, which caused a higher solubility of the copper fungicide than wheat flour, imparted a greater tenacity to the copper residue. From this it appears that, during rainfall, soya flour must prevent a rapid dissipation of the soluble copper.

Sorption Studies

A study on the performance of the protein-containing supplements in the presence of soluble copper showed that the materials tend to absorb

copper ions from the solution, and return them in the form of a complex. The extent of sorption of soluble copper by the supplements was determined by measuring the amount of copper in solution before and after treating the supplements with copper sulphate solution. It is apparent from the curves (Fig. 3) that the amount of copper sorbed by each supplement was dependent on the concentration of soluble copper until the sorption capacity of the supplement was reached. Further increase in the concentration of soluble copper did not cause an additional amount of copper to be sorbed.

A further study showed that the sorption capacity of soya protein was greater than that of soya flour, while in the case of wheat gluten and wheat flour an even greater relative increase in the sorption capacity was secured. This indicates that it is protein that forms the sorption complex with soluble copper. In other words, the retention of soluble copper apparently is caused by the formation of a copper-protein sorption complex. The sorption capacity of soya flour was found to be approximately 3 times that of wheat flour. Since the protein content of soya flour is also about 3 times that of wheat flour, it appears that the sorption capacity of the supplement is largely dependent upon its protein content. However, it is possible that the source of the protein, as well as its treatment during the manufacturing process, may influence the performance of these materials.

In the sorption tests, the saturated residues, which contained the sorption complexes formed by the various supplements, were filtered and washed to remove free copper sulphate. A portion of the filter cake was dried and analyzed for copper (Table 2). The availability of the copper in the resultant cake was determined by solubility tests.

Adherence tests were conducted on a portion of the undried filter cake that was redispersed in water. It was found that the tenacity coefficient of the soya flour sorption complex was 0.250, while that of the wheat flour sorption complex was 0.227. However, the magnitude of the deposit of the soya-flour complex was approximately 3 times that of the wheat-flour complex.

Due to the sorption capacity of the supplement for soluble copper, the amount of copper dissolved by the supplement must include both the free soluble copper and the copper sorbed. In other words, the solubility of the copper fungicide in suspension with the spray supplements (Table 2) represents the difference between the total dissolved copper and the amount of soluble copper sorbed by the supplement. In the copper fungicide-supplement suspension there apparently takes place: (1) solution of the copper fungicide; and (2) subsequent sorption of the soluble copper by the supplement until an equilibrium is reached. It should be noted that the amount of soluble copper, as determined at the point of equilibrium, is of particular value, since it furnishes information on the amount of available copper for the control of fungus disease on the one side, and on the safety of the spray on the other.

In practice, once the spray residue has become dry, the copper content of

the copper-protein complex and its solubility are factors in the availability of copper in the spray residue, as well as in the loss of copper by leaching. In this connection, the soya-flour complex has a higher copper content (1.76% Cu) than the wheat-flour complex (0.11% Cu), while the percentage of soluble copper in the soya-flour complex (14.3%) is less than in the wheat-flour complex (36.4%). The total amount of soluble copper from the soya-flour complex (2.5 mg. per gram) is, however, approximately 6 times higher than the soluble copper from the wheat-flour complex (0.4 mg. per g.). On the basis of the amount of soluble copper, it seems that the effectiveness of the soya-flour complex would be greater than that of the wheat-flour complex. Soya flour, due to its greater capacity for the sorption of soluble copper, may considerably reduce the loss by rainfall of copper released from the copper fungicide.

Field Tests

To determine the effect of the various supplements on the adherence of basic copper sulphate on the foliage, field tests were conducted at the Wilson

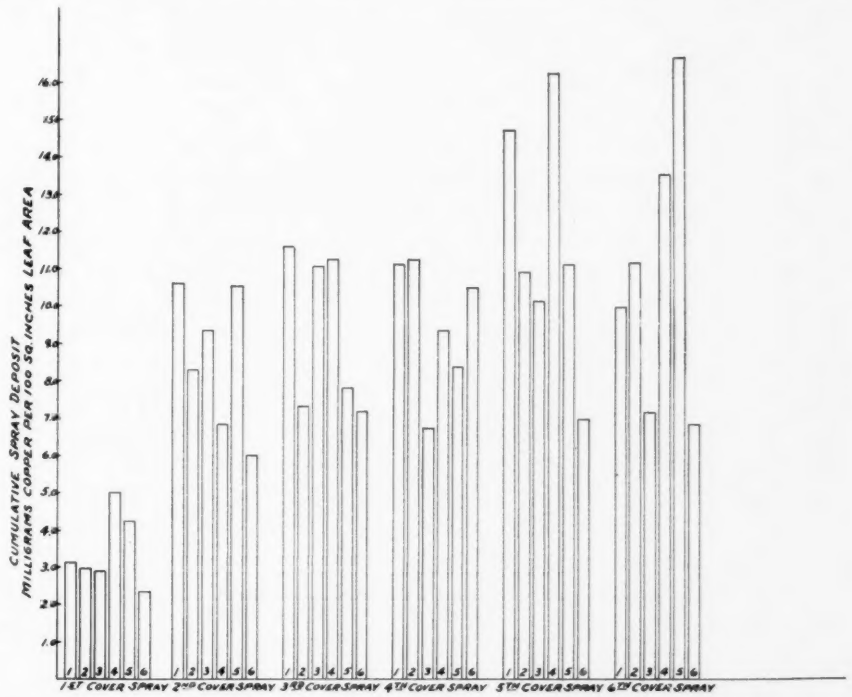

FIG. 4. Effect of spray supplements on the deposit of basic copper sulphate on apple foliage, 1939. 1, soya flour; 2, wheat flour; 3, calcium caseinate; 4, skim milk; 5, albumin; 6, check (no supplement).

apple orchard, Epworth, Ga. Three replicate plots, containing an average of 8 trees each, were laid out for each test. Six copper cover sprays were applied beginning 2 weeks after the petal fall application of lime-sulphur.

In order to compare the effect of the various supplements on the deposit and adherence of the copper fungicide, leaf samples were collected from each series of plots (4) and their spray residue analyzed quantitatively for copper (1). Care was taken to get representative samples of leaves from each plot and to prevent any loss of spray residue during subsequent treatment. Samples were collected directly after each spray application to determine the magnitude of the deposit. In 1939, samples were collected after an interval of weathering, to determine the tenacity of the spray deposit. In 1940, it was possible to collect samples at approximately weekly intervals, as well as immediately before and after each spray application. It was possible, in this manner to obtain a comprehensive comparison of the magnitude and tenacity of the spray deposit.

FIELD RESULTS AND OBSERVATIONS

The data from the orchard adherence tests are summarized in table 1 and illustrated in figures 4 and 5.

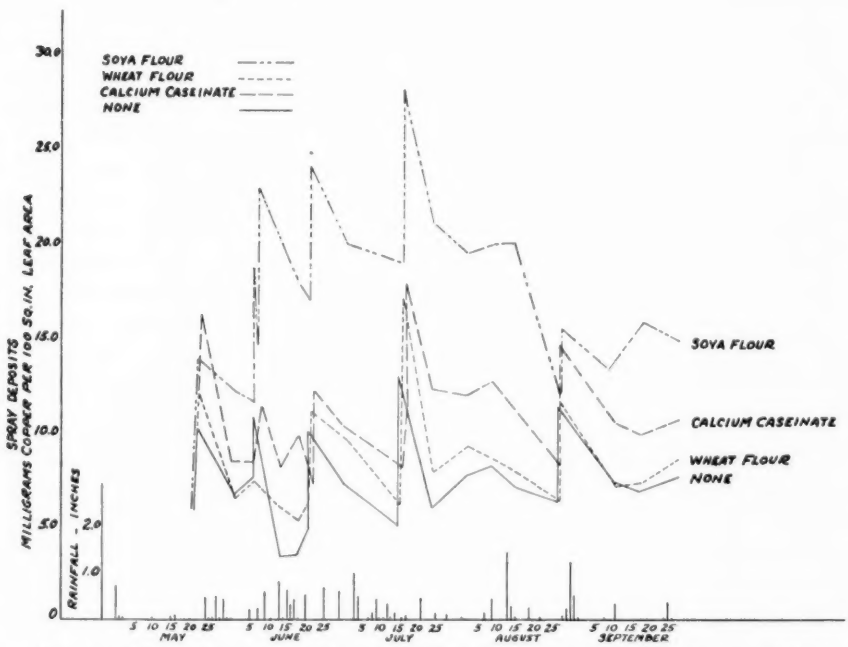

FIG. 5. Effect of spray supplements on the adherence of basic copper sulphate to apple foliage. 1940.

In the 1939 tests, the largest average deposit was obtained with skim milk, followed in decreasing order by soya flour, albumin, wheat flour, calcium caseinate and the check (no supplement). The largest average residue was obtained with skim milk, followed in order by soya flour, albumin, wheat flour, calcium caseinate and the check. The highest coefficient of tenacity was shown by sprays containing skim milk, followed in order by soya flour, albumin, calcium caseinate, wheat flour and the check.

In the 1940 tests, the largest average deposit was obtained with soya flour, followed in decreasing order by calcium caseinate, skim milk, wheat flour, check and albumin. The largest average residue was obtained with soya flour, followed in order by calcium caseinate, skim milk, wheat flour, check and albumin. The soya flour spray deposit had the highest coefficient of tenacity in 1940, followed by wheat flour, skim milk, calcium caseinate, albumin and the check.

The order of the comparative performance of the supplements in 1940 was somewhat different from their order in 1939. This can be attributed to the difference between the weather conditions and the amounts of rainfall during the two seasons.

The results obtained in the 1940 field tests show that there was a substantial difference between the magnitude of the spray residue obtained with soya flour and wheat flour. It should be emphasized that the magnitude of the residual spray deposit is just as important from the standpoint of sufficient coverage as the coefficient of tenacity of the residue. As an example, the tenacity coefficient of the 1940 sprays containing calcium caseinate was lower than that of sprays containing wheat flour, yet the amount of residual spray deposit was greater with calcium caseinate.

DISCUSSION

A study of laboratory and field results indicates that the spray supplements of the type included in these tests, function not only as spreaders and stickers for the spray deposit, but also as activators for the fixed copper fungicides. The supplements, when included in spray mixtures, exert a distinct dissolving action on the fixed copper fungicide, forming a sorption complex with the dissolved copper. This complex is an available source of copper in addition to the small quantity (1.5 p.p.m.) that is in solution.

The degree of activation of the basic copper sulphate appears to depend upon the protein content of the supplement. The effect of the protein content of the supplement can readily be seen from the superior performance of soya flour containing 53.5 per cent protein as compared with low protein wheat flour containing 8.6 per cent protein. These two flours are representative of high and low protein supplements, respectively.

The above indicates that a certain correlation exists between the dissolving action of the supplement upon the copper fungicide and the sorption of soluble copper by the supplement on the one hand, and the subsequent liberation of copper ion from the sorption complex on the other.

The sorption complex formed by the action of the supplement upon the fixed copper fungicide, plays a very important role in the adherence of the spray residue. The improved adherence obtained by the use of materials of high protein content as supplements, such as soya flour, can be partly attributed to the formation of this gelatinous sorption complex. The inferior adherence obtained with wheat flour probably is due to its low protein content and to the low amount of the sorption complex formed by this sup-

plement. The supplements that have a high sorption capacity, such as soya flour, increase the tenacity of the spray deposit to a greater extent than supplements of low sorption capacities, such as wheat flour. Apparently, a correlation exists between the sorption capacity of the supplements for soluble copper and the magnitude and tenacity of their respective spray residues.

From the above it seems that the improved adherence and activation of basic copper sulphate by the action of protein-containing supplementary materials may be readily utilized for the improved performance of fixed copper fungicides. Since the action of the supplements depends to a large extent on the proteins and their sources, it seems that the vegetable materials, such as soya bean protein, may be used advantageously to improve the performance of basic copper sulphate sprays, both from the standpoint of adhesiveness of the spray residue and from the activation of the copper fungicide.

SUMMARY

The performance of protein-containing spray supplements were studied from the standpoint of their effect upon the adherence and activation of fixed copper fungicides.

The results secured from spray tests conducted in the field and laboratory showed that:

The magnitude and tenacity of the spray residue are considerably improved by supplements containing protein.

Supplements, according to their protein content, exert a dissolving action on the fixed copper fungicide. The soluble copper released from the fixed copper fungicide is sorbed by the protein to form a gelatinous sorption complex, which increases the adhesiveness of the spray deposit.

A certain correlation exists between the dissolving action of the supplement upon the copper fungicide, and the subsequent liberation of soluble copper from the sorption complex.

A certain correlation exists between the sorption capacities of the supplements for soluble copper and the magnitude and tenacity of their respective spray residues.

COPPERHILL, TENNESSEE.

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POTATO-SCAB GARDENS IN THE UNITED STATES

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It has been known for some time that there are a comparatively large number of physiologic forms of *Actinomyces scabies* (Thaxter) Güssow, the organism that causes common scab in the potato. It has been observed, too, that the intensity of scab epidemics varies from year to year and from place to place, because of climatic and soil conditions. In any effort to breed scab-resistant varieties or to determine the genetic behavior of reactions to scab, physiologic forms and environmental factors must be given consideration. Varieties resistant to scab in one section of the country under a particular set of environmental conditions may be susceptible in another section. To determine to what extent a breeding program is affected by these sources of variation the cooperators in the National Potato Breeding Program in a conference at Ithaca, N. Y., in 1937, suggested that a number of uniform scab gardens be established in various sections of the country. Six such gardens were established in 1938—Elk River, Minn., Greeley, Colo., Lake City, Mich.,

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[5] The men who should be given credit for this study are the men who were responsible for growing the scab gardens. These men are:

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D. Reddick, " " " Ithaca, New York
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G. H. Rieman, " " " Madison, Wisconsin
R. W. Samson, " " " La Fayette, Indiana
G. H. Starr, " " " Laramie, Wyoming
E. J. Wheeler, " " " East Lansing, Michigan

Ithaca, N. Y., Kingston, R. I., and Presque Isle, Maine. In 1939 the number of gardens was increased by additional ones at Avon, Colo., Grand Rapids, Minn., Clear Lake, Iowa, Madison, Wisc., Manhattan, Kan., Baton Rouge, La., and La Fayette, Ind. In 1940 Shafter, Calif., Wooster, Ohio, Logan, Utah, and Laramie, Wyo., were added to the list.

MATERIALS AND METHODS

As a preliminary step in establishing these gardens it was suggested to the collaborators in the various State experiment stations where potato-breeding programs are in operation, that they send seed stocks of potato varieties they wished tested. Three varieties were received from F. A. Krantz of the Minnesota Agricultural Experiment Station and 11 from Donald Reddick of the New York Agricultural Experiment Station at Cornell University. In addition, 91 varieties that had shown various reactions to scab in the tests at Presque Isle, Maine, for the previous 2 years were assembled at Beltsville, Md., and distributed to the various stations.

Due to the small amount of seed stock available, only enough for duplicate rows of 5 hills of each could be sent to each station. This stock was planted in 1938 in rows adjacent to a row of a susceptible check variety. Scab data were secured by the various collaborators on each replicate and on its corresponding check. From the results obtained it was apparent that in addition to physiologic forms and environmental factors the individuality of the various persons who took the data was a source of variation. To eliminate this last factor as much as possible, the scabbiest tuber from each hill in the test with its corresponding check was sent to Beltsville, Md., in 1939, where the scab readings were all made by one person. In 1940 the data for the gardens in the eastern section of the United States were secured by one man, those for the Northwestern and Plateau States by another. These men had previously worked together so that the readings for that year are considered comparable. The method of taking the scab data in 1939 and in 1940 was similar to that described by Clark *et al.*[6] Two criteria were considered in judging the reactions of varieties and checks, *i.e.*, the type of scab pustule and the percentage of tuber surface covered with pustules.

Types of scab pustules were recorded in three categories: 1. Pustules relatively large and deep. 2. Pustules relatively large but superficial. 3. Pustules small and superficial.

The percentages of tuber surface covered with pustules were recorded in six classes:

Class	Extremes	Mean
	Per cent	*Per cent*
Trace	0–1	0.5
1	1–20	10.0
2	21–40	30.0
3	41–60	50.0
4	61–80	70.0
5	81–100	90.0

[6] Clark, C. F., F. J. Stevenson, and L. A. Schaal. The inheritance of scab resistance in certain crosses and selfed lines of potatoes. Phytopath. **28**: 878–890. 1938.

RESULTS

As the data for 1938 were obtained by a number of methods, they might be considered reliable in making the comparisons between varieties for a particular garden but not between varieties for all gardens. As the chief objective of the tests was to compare the behavior of the varieties in a series of gardens, the 1938 data are not used in this paper. The records for 1939 and 1940, on the other hand, were all secured by uniform procedure, and should be reliable for making comparisons within and between tests.

If the checks for both replications of a particular variety did not show 50 per cent of the tuber covered with No. 1 pustule type of scab, the data were not used in the final analyses. This was done to avoid pseudo-resistance. The standard is admittedly arbitrary and rather drastic, but if the readings on the varieties under test are to be considered dependable the checks must show the prevalence of a heavy epidemic.

The variation in scab incidence within gardens, between gardens, and from year to year, as determined by the susceptible check, greatly reduced the number of data that could be considered uniform. Twenty-two named and numbered varieties met the prescribed limits in both replications in 4 gardens and for 2 years. The data for these were used in the analyses of the variances of types of pustule, of percentages of tuber surface covered, and of the covariances between these two criteria.

PUSTULE TYPES

Type of pustule is considered by most investigators to be the more important criterion for judging the scab resistance of a potato variety. If large, deep pustules are found on the surface of the tubers the variety is considered susceptible, regardless of the percentage of tuber surface covered with the disease. The data for pustule types are given in table 1.

The checks all produced the No. 1 type of scab and are not recorded in the table. It is evident that some varieties are quite consistent in the character of scab pustule produced. Houma, the most susceptible variety in the list, showed the No. 1 type in all but one test. The exception was at Greeley, Colo., in 1939, where a No. 2 type of pustule was produced. Sebago showed a small degree of resistance with 10 records of No. 2 type and 6 of No. 1. It will be noted that in 2 tests of this variety one replication read No. 1, the other No. 2. Hindenburg with one exception produced No. 3 type. In Michigan in 1939 it produced No. 2 type. The reaction of Richter's Jubel was quite consistent with the No. 3 type in all except the 1940 Michigan test. In that test one replication read No. 1, the other No. 2. It is very unusual to find the No. 1 type on this variety. Arnica showed somewhat less resistance than either Hindenburg or Richter's Jubel, but a much higher degree of resistance than Houma, Sebago, or the susceptible checks. Certain of the unnamed varieties, such as AAO–9, 528–14, 528–119, 627–8, 627–213, and 1037–5, showed a relatively high degree of variation in the types of scab

produced. If Hindenburg is taken as the standard for resistance, and twice the standard error of a difference between means as the criterion of significance, then Richter's Jubel, 245–36, 256–11, 528–242, 627–7, and 627–164 are in the same class as Hindenburg so far as the type of pustule produced on the tubers in this series of tests is concerned. It should be noted that two of the resistant varieties had Richter's Jubel as a parent, one had Arnica, and two had Hindenburg.

TABLE 1.—*Data for type of scab pustules on 22 potato varieties grown in duplicate rows (A and B) in four gardens in 1939 and 1940*

Varieties	Presque Isle, Maine				Greeley, Colo.				Elk River, Minn.				Michigan				Mean of 16 tests
	1939		1940		1939		1940		1939		1940		1939		1940		
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
																	Per cent
Sebago	2[a]	2	1	1	2	2	2	2	1	2	2	2	2	1	1	1	1.6
Hindenburg	3	3	3	3	3	3	3	3	3	3	3	3	2	2	3	3	2.9
Richter's Jubel	3	3	3	3	3	3	3	3	3	3	3	2	3	3	1	2	2.8
Arnica	3	2	2	3	2	2	3	3	3	3	3	3	3	3	2	2	2.6
Houma	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1.1
AA0–9	1	2	1	1	3	3	3	3	2	2	2	2	2	1	1	1	1.9
245–36[b]	2	3	2	2	3	3	3	3	3	3	3	3	3	3	2	2	2.7
256–11	3	3	2	2	3	3	3	3	3	3	3	3	3	3	2	2	2.8
528–14	2	3	1	1	2	2	3	3	3	3	2	2	3	3	1	2	2.3
528–119	2	2	1	1	3	3	3	3	2	2	3	3	2	2	1	1	2.1
528–242	2	3	3	3	3	3	3	3	3	3	3	3	2	3	2	2	2.8
574–20	3	3	2	1	2	2	3	3	2	2	3	2	2	2	1	2	2.2
627–7	3	3	2	2	3	3	3	3	3	2	3	3	3	3	2	2	2.7
627–8	3	2	1	1	2	2	3	3	3	3	2	2	1	2	1	2	2.1
627–18	2	3	1	2	3	3	3	3	2	3	2	2	3	3	2	2	2.4
627–103	3	3	2	2	2	2	3	3	2	3	3	3	3	3	1	1	2.4
627–126	2	2	1	1	2	2	3	3	3	2	3	3	3	3	2	2	2.3
627–164	2	3	2	2	3	3	3	3	3	3	3	3	3	3	2	2	2.7
627–210	3	2	2	1	3	3	3	2	2	2	2	3	3	3	2	2	2.4
627–213	2	2	2	3	3	3	2	2	2	2	2	2	1	3	1	1	2.1
627–273	2	2	2	2	3	3	3	3	3	2	2	3	2	2	1	2	2.3
1037–5	2	2	1	2	3	3	1	1	1	1	1	1	1	1	1	1	1.4
Difference for significance at 5-per cent level																	0.2

[a] Types of pustule:
1 = Relatively large and deep.
2 = " " but superficial.
3 = " small and superficial.

[b] Parents of the crosses represented in the table are:
245, 444–12 × Richter's Jubel
256, 444–12 × Arnica
528, Richter's Jubel × 44537
574, Arnica × 44537
627, Hindenburg × Katahdin
1037, 45208 × 44537

PERCENTAGES OF TUBER SURFACE COVERED WITH SCAB

The data for percentage of tuber surface covered with scab pustules are given in table 2. The susceptible checks were not included in the table, since the tubers of these were all heavily infested—none of them showing less than 50 per cent of their surface covered with scab.

TABLE 2.—*Percentage of tuber surface covered with scab pustules on 22 potato varieties grown in duplicate rows (A and B) in four gardens in 1939 and 1940*[a]

Varieties	Presque Isle, Maine				Greeley, Colo.				Elk River, Minn.				Michigan				Mean of 16 tests
	1939		1940		1939		1940		1939		1940		1939		1940		
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
																	Per cent
Sebago	50.0	50.0	70.0	50.0	30.0	30.0	10.0	10.0	30.0	10.0	30.0	10.0	50.0	30.0	50.0	30.0	33.8
Hindenburg	0.5	10.0	10.0	10.0	0.5	0.5	10.0	10.0	10.0	0.5	0.5	0.5	10.0	10.0	0.5	0.5	5.3
Richter's Jubel	0.5	10.0	0.5	0.5	10.0	10.0	10.0	10.0	0.5	0.5	0.5	0.5	10.0	0.5	0.5	10.0	4.7
Arnica	0.5	0.5	0.5	0.5	50.0	10.0	10.0	0.5	0.5	0.5	0.5	0.5	30.0	50.0	0.5	50.0	12.8
Houma	70.0	70.0	50.0	70.0	10.0	10.0	50.0	50.0	50.0	50.0	10.0	30.0	70.0	50.0	70.0	90.0	50.0
AA0–9	30.0	50.0	50.0	50.0	50.0	50.0	30.0	30.0	10.0	50.0	30.0	30.0	30.0	30.0	50.0	30.0	37.5
245–36	30.0	50.0	10.0	30.0	10.0	0.5	30.0	0.5	10.0	10.0	0.5	0.5	50.0	30.0	30.0	10.0	18.9
256–11	30.0	10.0	10.0	10.0	10.0	10.0	30.0	0.5	0.5	0.5	0.5	0.5	50.0	50.0	10.0	10.0	14.5
528–14	30.0	30.0	50.0	50.0	0.5	10.0	0.5	10.0	10.0	30.0	10.0	0.5	30.0	50.0	10.0	10.0	20.7
528–119	10.0	30.0	10.0	10.0	10.0	0.5	10.0	10.0	10.0	0.5	0.5	0.5	70.0	30.0	50.0	70.0	20.1
528–242	30.0	10.0	0.5	0.5	0.5	0.5	30.0	10.0	0.5	0.5	10.0	10.0	50.0	30.0	30.0	10.0	13.9
574–20	30.0	10.0	50.0	10.0	10.0	0.5	10.0	0.5	30.0	30.0	30.0	0.5	50.0	30.0	10.0	50.0	22.0
627–7	30.0	30.0	10.0	10.0	0.5	0.5	10.0	10.0	0.5	0.5	0.5	0.5	10.0	10.0	10.0	10.0	8.9
627–8	10.0	30.0	10.0	10.0	30.0	10.0	30.0	0.5	0.5	0.5	10.0	0.5	10.0	30.0	10.0	10.0	12.6
627–18	10.0	10.0	10.0	10.0	0.5	0.5	30.0	0.5	30.0	0.5	30.0	0.5	10.0	10.0	10.0	30.0	12.0
627–103	0.5	10.0	10.0	10.0	50.0	10.0	0.5	0.5	10.0	0.5	0.5	10.0	0.5	0.5	10.0	10.0	8.3
627–126	30.0	10.0	10.0	10.0	10.0	10.0	0.5	0.5	0.5	10.0	0.5	30.0	10.0	10.0	10.0	0.5	9.5
627–164	0.5	0.5	10.0	0.5	0.5	0.5	10.0	10.0	0.5	0.5	0.5	0.5	0.5	10.0	0.5	10.0	3.5
627–210	10.0	30.0	10.0	30.0	0.5	30.0	0.5	10.0	10.0	10.0	10.0	0.5	30.0	30.0	10.0	10.0	14.5
627–213	30.0	50.0	30.0	10.0	0.5	0.5	0.5	10.0	10.0	10.0	10.0	10.0	30.0	50.0	30.0	50.0	20.7
627–273	50.0	30.0	30.0	30.0	0.5	50.0	0.5	0.5	0.5	10.0	10.0	0.5	50.0	50.0	50.0	30.0	24.5
1037–5	10.0	0.5	10.0	10.0	0.5	0.5	30.0	10.0	70.0	50.0	10.0	10.0	50.0	50.0	50.0	50.0	25.7
Difference for significance at 5-per cent level																	7.6

[a] Percentages of surface covered were taken in 6 classes. The data in the table are given in mean percentage.

Class	Extremes	Mean
	Per cent	*Per cent*
Trace	0–1	0.5
1	1–20	10.0
2	21–40	30.0
3	41–60	50.0
4	61–80	70.0
5	81–100	90.0

If the resistance of the varieties in these tests were judged by the data in table 2 the conclusions would be somewhat similar, but would not agree in every detail with those drawn from the data on type of pustule. Both Houma and Sebago showed a much greater variation in percentage tuber surface covered than they did in type of pustule. This was true also for the unnamed seedling varieties that might be considered intermediate in resistance, such as 574–20. The resistant varieties were more consistent.

TABLE 3.—*Variance for type of pustule, percentage of tuber surface covered, and the correlations between the two criteria*

Sources	d/f	Type of pustule	Percentage of tuber surface covered	Type of pustule and tuber surface covered
		Variances	*Variances*	*Correlation coefficients*
Varieties	21	3.40†	2,073.57†	−.85†
Replications	1	0.34	159.57	−.99†
Places	3	8.80†	5,721.35†	−.90†
Years	1	8.59†	1,092.04†	−.99†
Varieties × places	63	0.37†	425.51†	−.33†
Varieties × years	21	0.22*	96.25	−.03
Places × years	3	4.07†	156.10	−.47†
Varieties × places × years	63	0.46†	235.99†	−.38†
Error	175	0.11	118.37	.....

* = Significant.
† = Highly significant.

Again taking Hindenburg as the standard for resistance and twice the standard error of a difference between means as indicating significance, Hindenburg, Richter's Jubel, Arnica, and selections 7, 8, 18, 103, 126, and 164 from the cross 627, are in the same class. It should be noted that only 4 of these—Hindenburg, Richter's Jubel, 627–7, and 627–164—were found in the most resistant class when judged by the type of pustule produced.

VARIANCES AND CORRELATIONS

The variances for type of pustule and percentage of tuber surface covered, and the correlations between these two criteria, are given in table 3. There was a highly significant difference between the means of the pustule types for varieties, places, and years, but the variance for replication was not significant. The interactions for variety × place and place × year were highly significant also, but the variance for variety × year did not greatly exceed the 5-per cent level. A comparison of the variances for percentage of tuber surface covered indicated highly significant differences for varieties, places, and years, but not for replications. Among the first order interactions only the variance for variety × place was significant.

Highly significant correlation coefficients were found between type of pustule and percentage of tuber surface covered for all but one of the sources of variability calculated.

DISCUSSION

It is evident that there are very significant differences between varieties of potatoes in their reactions to scab infection, and that comparative differences were fairly constant for the 22 varieties tested in 4 widely separated gardens. This might indicate that physiologic forms of *Actinomyces scabies* did not play a very important part in these tests. The significant interactions between variety and place would indicate, however, either physiologic forms or the influence of other environmental factors or both. The high interaction between place and year for type of pustule is evidence in favor of environmental factors rather than different physiologic forms, as it is highly improbable that the population of scab organisms would change its genetic nature from one year to the next.

Varieties selected in Maine showing the resistance of Hindenburg or Richter's Jubel have a good chance of being resistant in other parts of the country, but, because of the probability of the presence of physiologic forms and of the certainty of environmental influences, they should be tested in a wide range of conditions and over a period of years before generalized statements concerning their reactions are made.

SUMMARY

The 22 potato varieties tested in 4 potato-scab gardens show highly significant differences in their reactions to the common scab organisms for pustule-type and tuber-surface coverage.

The type of pustule and the percentage of tuber surface covered with pustules were highly correlated, indicating that in the majority of cases the relative reactions of the varieties to scab infection could be estimated by either criteria.

In general, the varietal reactions were quite constant for the 2 years and for 4 places. The highly resistant varieties showed less tendency to vary between years or between places than the slightly resistant or susceptible ones.

The interactions between variety and place are significant and could be due to environmental factors or to physiologic forms or to both. The highly significant interaction for pustule type between place and year indicates the influence of environment rather than different physiologic forms.

From the potato breeding point of view it seems possible to select varieties highly resistant to common scab in Maine and have them show similar resistance in other sections of the country, but before generalized statements of their reactions are made, they should be tested in a wide range of environmental conditions.

EFFECT OF ENVIRONMENT ON THE PREVALENCE OF SOIL-BORNE RHIZOCTONIA[1]

O. H. Elmer

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The rhizoctonia disease of potatoes, caused by *Corticium vagum* B. and C., attacks the potato plant through soil-borne mycelium or mycelium produced from soil-borne or tuber-borne sclerotia. Under eastern Kansas conditions this disease is characterized by local necrotic lesions on underground stems and stolons. A decrease in yield results from these lesions and from the delayed emergence and, consequently, the shortened growing season caused by the rotting off of infected sprouts prior to their emergence. The growing season for the spring potato crop in eastern Kansas ends in early July because the temperature is then so high as to stop growth and the plants die.

Tuber-borne Rhizoctonia sclerotia generally are recognized as being the more frequent source of infection of the potato. Prior to and in the early years of this investigation, evidence was obtained indicating that considerable damage to potatoes also may be caused by soil-borne Rhizoctonia.

The object of the investigations herein reported was to determine the relative importance of soil-borne Rhizoctonia as a cause of injury to the potato plant. Studies were made on the effect of soil moisture, including the drying effect of temperature and wind, on the ability of Rhizoctonia to persist from year to year in potato field soils.

METHODS

The prevalence of Rhizoctonia in potato-field soils was measured by the frequency with which soil-borne Rhizoctonia produced infections to experimental potato plants. The potato plants used in this investigation were produced from rhizoctonia-sclerotia-free tubers that had in addition been treated with an effective seed-potato treatment. Seed-potato treatments consisted of a 5-minute soak in hot formaldehyde solution, a 90-minute soak in 0.1-per cent solution of bichloride of mercury, or a 10-minute soak in a 0.2-per cent solution of bichloride of mercury acidulated with one per cent hydrochloric acid. Rhizoctonia infections that developed on potato plants produced from the treated sclerotia-free tubers were believed to have been initiated by the soil-borne organism. The prevalence of such infections is assumed to serve as an index on the prevalence of Rhizoctonia in the field soils.

Experimental plantings of sclerotia-free, treated potatoes in commercial fields were made anually during the seasons 1928 to 1940, inclusive. The seed pieces were planted in duplicated 50-foot rows in March during plant-

[1] Contribution No. 414, from the Department of Botany, Kansas Agricultural Experiment Station.

ing of the commercial crop. The soil-rhizoctonia test plots were established in 3 commercial fields in 1928, and thereafter in an average of 15 such fields.

During the first 4 years of this investigation rhizoctonia-infection data were obtained when the potatoes were harvested in July, but difficulty was experienced in ability definitely to recognize rhizoctonia lesions at this time, because the plants were reaching maturity and some of them were dying. In 1932 and thereafter, rhizoctonia-infection data were obtained in May when the plants were beginning to bloom. Maximum rhizoctonia infection, including infections on underground stems and stolons, has occurred by this time, and the lesions can be recognized easily. In obtaining these data the plants, including the newly developing tubers, were removed from the soil and the infection data were recorded.

During the seasons 1931 to 1940 rhizoctonia-sclerotia-infected nontreated tubers were planted in rows adjoining the rhizoctonia-free potatoes. The infections that developed on these control plants served as an index on the ability of Rhizoctonia to produce lesions on potato plants under the prevailing environmental conditions.

Weather records from 1927 to 1939 showing the factors affecting soil-moisture content of potato fields in eastern Kansas are presented in this report. Special attention has been given to annual precipitation and to rainfall, temperature, and wind during July and August. These weather conditions are of primary importance in determining the soil-moisture content of the soils of commercial eastern Kansas potato fields during July and August, when soil-moisture content becomes the lowest.

RESULTS

The results obtained during the 13 years of this investigation show that environmental conditions are highly important in determining how successfully soil-borne Rhizoctonia can persist from year to year in eastern Kansas field soils.

The commercial potato crop in eastern Kansas matures and usually is harvested in early July. Throughout the rest of the summer the fields usually remain fallow. Summer temperature in this area is too high for the production of rhizoctonia sclerotia as is disclosed by the fact that they are rarely found here on tubers of the early summer crop. Thereafter the fungus must consequently exist saprophytically as soil-borne mycelium. In this state its persistence depends on the presence of sufficient soil moisture to prevent death from desiccation. The springtime prevalence of soil-borne Rhizoctonia in these fallow fields indicates to what extent this fungus is able to persist in such field soils during the preceding summer.

A second crop of potatoes or a crop of rhizoctonia-susceptible vetch, cowpeas, or soybeans, sometimes planted about August 1, promotes the ability of this organism to persist in potato fields during hot, dry summers.

Effect of Soil Moisture Content During the Summer on Survival of Rhizoctonia in the Soil

The soil-moisture content of fallow potato fields during the summer

months is largely determined by the total annual precipitation and the July-August rainfall, by summer temperatures, and by air movement, which may be measured as total miles of wind per month. Records of these environmental factors for 1927 to 1939 inclusive, are presented in table 1. The summer temperatures recorded are the average daily maxima for the month.

TABLE 1.—*Effect of environment on prevalence of soil-borne Rhizoctonia*

Year	Average maximum daily temperature (degrees F.)		Precipitation (inches)			Wind miles			Per cent[a] infection
	July	August	July	August	Annual	July	August	Total	
1927	86.7	80.0	6.23	6.80	43.80	5,309	4,737	10,046	.....
1928	87.8	87.6	2.75	4.91	32.20	5,330	5,454	10,784	53.2
1929	88.6	89.5	1.70	2.64	32.81	5,695	5,297	10,992	50.4
1930	93.9	90.9	1.57	2.88	33.89	6,035	5,089	11,124	21.3
1931	91.8	85.0	3.41	6.48	36.79	5,394	4,757	10,151	36.2
1932	90.6	89.4	2.89	7.82	28.36	6,314	6,314	12,628	32.1
1933	92.9	86.3	2.47	3.50	22.24	6,364	5,869	12,233	12.5
1934	102.0	97.0	2.87	1.71	27.30	6,907	6,548	13,455	3.0
1935	99.7	91.6	0.03	3.98	37.31	6,205	6,160	12,365	1.1
1936	102.5	101.2	1.11	0.57	22.63	6,599	6,544	13,143	4.9
1937	95.5	96.4	1.65	3.51	19.43	6,287	6,384	12,671	3.1
1938	94.0	96.2	3.97	2.15	29.01	5,610	7,299	12,909	1.1
1939	99.1	89.3	1.26	3.51	22.33	6,759	6,198	12,957	1.3
1940	.....	.....	.....	.....	.....	.....	.....	.....	3.8

[a] From Rhizoctonia that persisted in the soil through the preceding summer.

Meteorological data from the Topeka station of the United States Weather Bureau, presented in table 1, show that the annual precipitation and the July–August rainfall were relatively high and that temperature and total wind mileage were relatively low during July and August of 1927 to 1932, inclusive.

The annual rainfall at Topeka for the period 1927 to 1932, inclusive was respectively, 43.80, 32.20, 32.81, 33.89, 36.78, and 28.36 inches. The July–August rainfall during these years was, respectively, 13.03, 7.66, 4.34, 4.45, 9.89, and 10.71 inches. The maximum daily temperature for July and August averaged below 90° F., and total miles of wind for the same months varied from 10,046 to 11,124 miles.

As a result of these environmental conditions, eastern Kansas potato-field soils did not become excessively dry and a considerable amount of soil-borne Rhizoctonia was able to persist during the summer months. It is shown in table 1 that 53.2, 50.4, 21.3, 36.2, 32.1, and 12.5 per cent, respectively, of the experimental potato plants became infected with Rhizoctonia during the springs of 1928 to 1933.

Weather conditions in eastern Kansas during 1933 to 1939, inclusive, differed widely from those of the first 6 years of this investigation. Annual precipitation during 1933 to 1939, inclusive, with the exception of 1935 was relatively low; only small amounts of rain fell during the summer months of these years. The annual rainfall during these 7 years was, respectively,

22.24, 27.30, 37.31, 22.63, 19.43, 29.01, and 22.33 inches. Rainfall during July and August of each of these years was, respectively, 5.97, 4.58, 4.01, 1.68, 5.00, 6.12, and 4.77 inches. Summer temperatures were relatively high during these years, and there was considerably more wind than during the first 6 years of this investigation. The average daily maximum temperature for July and August in 1933 to 1939, respectively, was 89.6, 99.5, 95.6, 101.9, 95.9, 95.1, and 94.2° F., and total miles of wind during July and August of these years varied from 12,233 to 13,455 miles.

As a result of the deficient rainfall, the high summer temperatures, and the relatively windy weather during 1933–1939, field soils became excessively dry. Sub-soil moistures became depleted during the consecutive years of drought and the meager rains that fell soon evaporated due to the then prevalent dry weather conditions.

Infection data obtained during the springs of 1934 to 1940, inclusive, (table 1) indicate that very little soil-borne Rhizoctonia was able to survive in eastern Kansas potato fields during the summers of 1933 to 1939. The average number of potato plants infected by soil-borne Rhizoctonia during the succeeding springs of 1934 to 1940 was, respectively, 3.0, 1.1, 4.9, 3.1, 1.1, 1.3, and 3.8 per cent.

Sclerotia-infected, nontreated potatoes, planted as controls in rows adjoining the sclerotia-free treated tubers, produced many rhizoctonia-infected plants. The percentages of plants that became infected with the seed-borne fungus in the tests of 1930 to 1939 were, respectively, 85.5, 95.6, 92.2, 88.6, 94.4, 90.7, 96.4, 99.3, and 92.3. These data provide evidence that the low infection percentages by soil-borne Rhizoctonia during the later years of this investigation were not due to unfavorable environmental conditions but to the presence of only small amounts of Rhizoctonia in the field soils.

Effect of Living Host Plants on Survival of Rhizoctonia in the Soil

Data obtained during this investigation indicate that the survival of soil-borne Rhizoctonia in relatively dry soils is greatly facilitated when living host plants of this pathogen are present.

It was repeatedly noted that the Rhizoctonia content of potato field soils was higher when a fall crop of potatoes or when such rhizoctonia-susceptible fertility crops as vetch, or cowpeas, had been grown in those fields the preceding summer and fall. Sclerotia-free, treated tubers were planted in the spring of 1936 in 3 fields in which a fall crop of potatoes had been produced in 1935. Soil-borne Rhizoctonia developed the following spring on, respectively, 15.0, 17.5, and 58.3 per cent of the experimental potato plants in these 3 fields. In contrast, only 4.9 per cent infection occurred in those commercial fields that, in 1935, had not produced a fall crop of potatoes. The field in which 58.3 per cent infection occurred had been planted in August, 1935, with untreated tubers severely infected with rhizoctonia sclerotia.

DISCUSSION

Prevalence of soil-borne Rhizoctonia in the potato fields of the Kansas

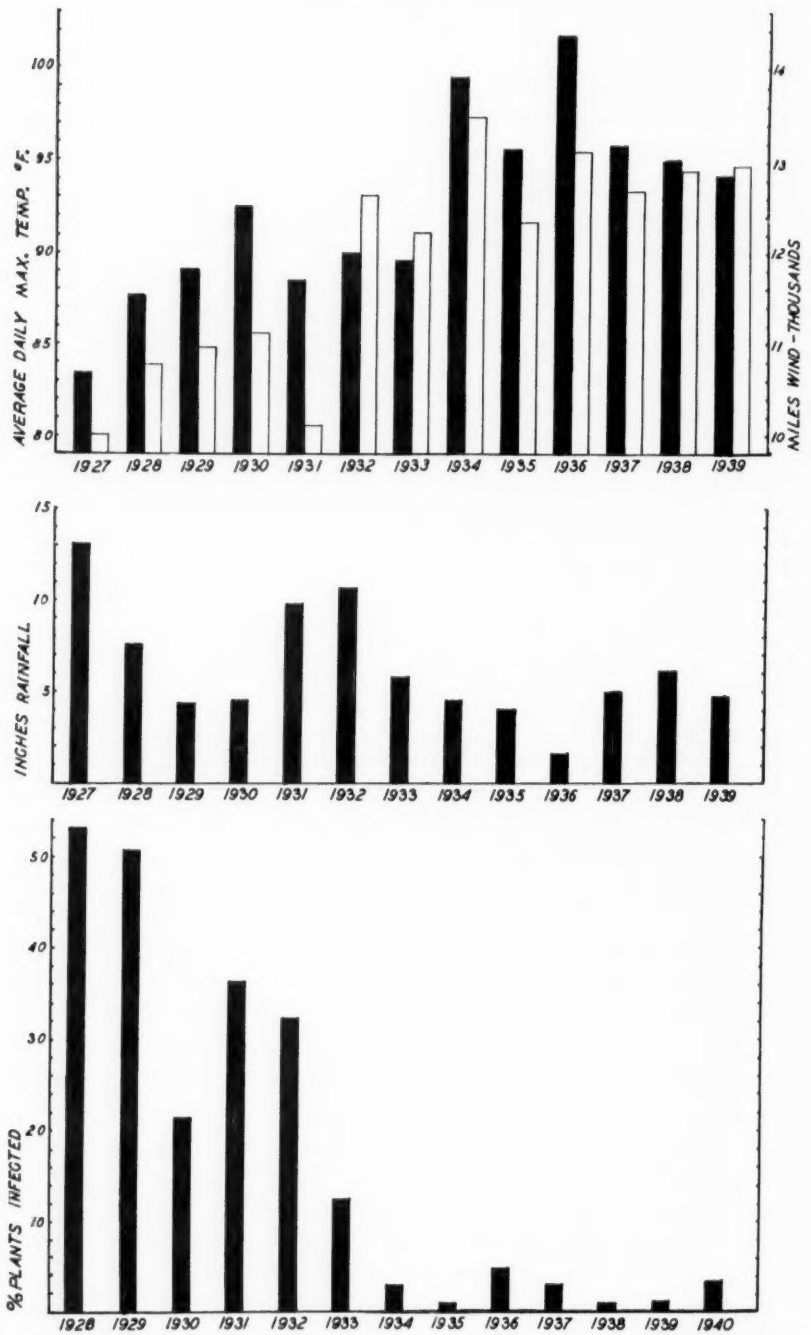

FIG. 1. Rainfall, temperature, and wind during July and August and subsequent prevalence of soil-borne Rhizoctonia. The graph indicates the relatively high rainfall and the relatively low temperatures (solid bar) and wind (light bar) during July–August, 1927–1932, when much Rhizoctonia was able to persist in potato field soils. Relatively low rainfall, high temperature, and windy weather during July–August, 1933–1939, were followed by a greatly reduced rhizoctonia content of the field soils.

River Valley varied greatly during the 13 years of this investigation. The data indicate that, under eastern Kansas conditions, Rhizoctonia can persist saprophytically in fallow soil only during summers when the soil does not become excessively dry.

The correlation between summer weather conditions and the survival ability of Rhizoctonia in potato-field soils is illustrated in figure 1. This graph illustrates how the abundant summer rainfall of the early years of this investigation were accompanied by low summer temperature and relatively little wind, and, inversely, how low rainfall in July and August of the later years was accompanied by high summer temperature and high winds. The graph further illustrates how soil-borne Rhizoctonia was comparatively abundant following moist and relatively cool summers and how its prevalence decreased following summers when the field soil became excessively dry.

The periodic dying out of soil-borne Rhizoctonia, induced by environmental conditions, is important to potato growers in eastern Kansas. Infections from soil-borne Rhizoctonia may be prevalent in potato plants following such cool, moist summers as those of 1927 and 1928, but injury from this source is of little importance following summers of low soil-moisture content. The prevalence of soil-borne Rhizoctonia in eastern Kansas can be approximately predicted from the relative soil moisture content of the preceding summer.

The potato growers' most serious losses from Rhizoctonia usually result from seed-borne inoculum. Observations during the course of this investigation indicated that severity of injury to affected plants is much the same, whether from tuber-borne or soil-borne Rhizoctonia, but that the infections from the seed-borne organisms are usually more prevalent. In regions like eastern Kansas, where soil-borne Rhizoctonia may periodically be practically eradicated because of environment, effective seed-potato treatments are valuable not only in preventing infection of the current season's crop but also in preventing reinfestation of the soil with this fungus.

SUMMARY

Wide differences were noted in the prevalence of soil-borne Rhizoctonia in Kansas potato fields during the 13 years of this investigation. Rhizoctonia was able to survive in the soil only in those years when there was sufficient summer rainfall to prevent desiccation and death of the organism in its mycelial stage. Sclerotia were absent during the critical summer months, because their formation requires cooler weather than usually occurs in Kansas in July and August.

The rhizoctonia organism can persist parasitically on infected host plants during summers when the soil is so dry that it cannot survive saprophytically.

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CONTROL OF FUNGI IN MUSHROOM CASING SOIL BY STERILIZATION WITH CHLOROPICRIN[1]

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INTRODUCTION

Mushroom beds are covered with soil 1 inch deep in order to induce the formation of mushrooms. An increasing number of growers are employing soil sterilization as a regular part of their control practice. Sterilization of this soil (casing soil) is aimed principally at eradication of *Mycogone perniciosa* Magnus, the organism causing the destructive "bubble" disease. This organism has become so widespread in areas where mushrooms have been grown intensively that it is almost impossible to obtain soil that is entirely free of it. Soil sterilization has been particularly necessary for growers who have mushrooms on the beds in early fall and late spring when the "bubble" disease becomes most serious.

Increased attention to the control of mushroom diseases has indicated that the casing soil may also be the avenue of entrance for other organisms besides *Mycogone perniciosa*. This is particularly true of soils contaminated with trash or drainage from mushroom houses. Among the organisms that may be introduced with contaminated soil are the following: *Verticillium malthousei* Ware, causing spotting and malformation of the mushrooms: *Dactylium dendroides* (Bull.) Fr., causing soft decay; *Pseudobalsamia microspora* Diehl and Lambert, the "truffle," which may be introduced into the compost when infested soil is added to it or which may be carried in with the casing soil (1). The less important green molds *Trichoderma* sp. and *Penicillium* sp., which may become serious if the beds are not properly managed, are also common inhabitants of the soil.

Under certain conditions the opportunity exists for pests like mites and springtails to find their way into the houses via the casing soil. Infestations of the long-legged mite, *Linopodes antennaepes* Banks have been traced to the casing soil. The frequency with which such pests get into the houses by this means is not definitely known, yet the possibility must be considered in a strict sanitation program.

In the widest sense, then, soil sterilization should be regarded as having the general aim of eradicating all the injurious fungi and pests. It must be admitted, however, that, ordinarily, the miticidal and insecticidal properties of the sterilization treatment are unimportant.

Steam sterilization has proved satisfactory as a fungicidal as well as an insecticidal measure. Its chief disadvantages to most growers are cost of equipment and necessity of hiring it. Very infrequently certain soils are damaged by the steam treatment. This toxic effect also has been observed

[1] The writer is indebted to J. W. Sinden and C. A. Thomas of the Pennsylvania State College for suggestions in the course of the study and for criticism of the manuscript.

in greenhouse soils. Lambert has pointed out the danger of overheating casing soil (5).

Beach has recommended sterilization with formaldehyde (1). The necessary technique is simple and inexpensive, and is very effective against *Mycogone perniciosa*. Consequently, it has become widely popular among mushroom growers. Formaldehyde is not an insecticide, though, admittedly, this is not of prime importance. Some soils, particularly those in which nitrogen-fixing plants have been grown, respond unfavorably to formaldehyde treatment. In these cases, the soil becomes predisposed to the development of an excessive amount of green molds or the mushrooms fail to form properly. This type of damage can, however, be avoided by using the proper kind of soil. Formalin is now somewhat difficult to secure because of war priorities.

The success of chloropicrin as a nematocide, insecticide, and fungicide has suggested its use for mushroom casing soil (2, 3, 7). It volatilizes rapidly into a powerfully lethal "tear gas." Accordingly, research was undertaken to determine its possible efficacy in sterilizing casing soil.

EXPERIMENTAL CONTROL OF FUNGI IN CASING SOIL

Since eradication of the spores of *Mycogone perniciosa* is the primary purpose of sterilization, several studies were made on the susceptibility of this organism to chloropicrin. Four cubic feet of soil were inoculated with *Mycogone* spores by mixing in macerated tissue of a large number of mushrooms badly affected with "bubble" disease. This soil was divided into 4 boxes, each holding 1 cu. ft. Three of the boxes were treated with chloropicrin at the rate of 2, 3 and 5 ml., respectively. A single injection, 6 in. deep, was made in the center of each box. One box was left untreated. The surface of all the boxes was watered, then covered with several layers of cardboard. After 3 days the cover was removed. The gas was still very strong at this time in the treated boxes. In 10 days all trace of it had disappeared. The contents of each box was used to case 2 duplicate trays, each containing about 7 sq. ft. of mushroom bed. The trays were then put under ordinary cold frames in preparation for bearing. The experiment was conducted in the spring, and the afternoon temperature in the frames was frequently as high as 70° F.

On the first break all the mushrooms on the untreated plots were severely malformed, showing the characteristic puffball-like symptom induced by *Mycogone perniciosa*. On the remaining 6 plots no diseased mushrooms appeared. The infected plots were immediately discarded. Subsequently, mushrooms were picked off the remaining plots for the next month. No diseased mushrooms appeared on any of the plots.

It is evident that under the conditions of the experiment 2 ml. of chloropicrin per cu. ft. sufficed to kill the spores of *Mycogone perniciosa*. All the treated plots seemed to react in the same way. A good number of high-quality mushrooms were picked from each tray.

The effect of chloropicrin on other fungi common to mushroom beds was

similarly determined. Soil was inoculated with spores of *Verticillium malthousei, Dactylium dendroides,* and *Pseudobalsamia microspora.* Two trays were used for each species, one containing soil treated at the rate of 2 ml. of chloropicrin per cu. ft. and the other untreated.

The untreated plot containing *Verticillium* spores produced mushrooms that were about 80 per cent spotted on the first break. No spotted mushrooms appeared in the treated soil. It is interesting to note that Godfrey has reported control of *Verticillium alboatrum* on strawberries and Verticillium wilt of tomatoes with chloropicrin (3). The untreated tray in which infection occurred was covered with glass to increase the humidity. On the next break all the mushrooms were spotted or malformed. The control was disease-free.

No sign of the *Dactylium* appeared in the untreated plot until the second break, when it appeared in the corner of the plot. The tray was then heavily watered and covered with glass. In 2 weeks' time the entire tray was covered with a luxuriant growth of Dactylium mycelium, and mushrooms that emerged were attacked. The fungus did not appear in the treated tray.

In the trays cased with soil inoculated with *Pseudobalsamia,* truffle did not appear either in the treated or non-treated plot. Hence, no conclusion can be drawn.

FUMIGATION OF CASING SOIL IN BINS

For large-scale sterilization it was decided to treat the soil in roughly constructed bins erected on the composting ground in the rear of the mushroom houses (Fig. 1). Such a procedure should secure a uniform distribu-

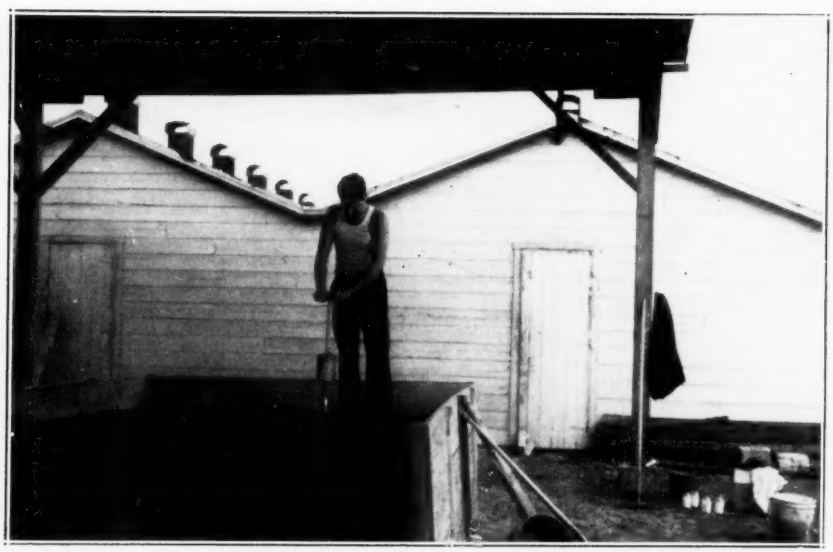

FIG. 1. Injection of chloropicrin into casing soil in wooden bin.

tion of the chloropicrin and guarantee the best possible confinement of the gas. A bin approximately 9 ft. wide, 11½ ft. long and 3 ft. high, holding

enough soil to case a standard house of about 4000 sq. ft. of bed space, was made with the sides detachable so that they could be broken down and either transported or stored. Wooden or iron supports were propped against the sides when the bin was full. The bin was erected immediately after the houses were filled.

An interval of 3 to 4 weeks ordinarily intervenes between filling and casing, ample time to allow escape of the gas. As usual, the soil was screened through a 1-in.-mesh screen before placing in the bin. A bottom layer of soil 1 ft. deep was thrown into the bin, leveled off, and marked out into 1 ft. squares. Five ml. of chloropicrin were injected 4 inches deep into the center of each square. A second and third layer of soil, each 1 ft. deep, were treated in the same manner. About 5½ lb. of chloropicrin were used for the bin. When the latter was full the surface was watered to a depth of about an inch and covered with the glue-coated paper recommended by Godfrey (3). After 3 days the cover was removed. A week later the pile was turned over. Subsequent experiments proved this operation unnecessary, since the gas normally escaped within 2 weeks.

The soil used in this experiment came from a field adjoining a mushroom plant. The grower in whose houses the experiments were done had used soil from the same source for years and had finally been compelled to sterilize it because of serious losses from the "bubble" disease.[2] A binful of this naturally infested soil was treated in the above manner. A second pile was left untreated. A third was treated with formaldehyde, as recommended by Beach, and a fourth was steam-treated. Four houses were subsequently cased with the 4 piles treated in different ways. The houses were kept in bearing 3 months, during which time careful observations were made. Many bubbles appeared on the first break in the house containing non-treated soil. On subsequent breaks the loss resulting from Mycogone infection became quite serious. An unusually long hot spell increased the severity of infection, so that the difference between this house and the houses containing treated soils was very conspicuous. In these practically no bubbles appeared in the first 3 weeks. A few appeared in later breaks, evidently because of reinfection. Apparently all 3 of the treatments, chloropicrin, steam, and formaldehyde, were equally effective. While production records were not kept in these houses, it was impossible to observe differences in the variously treated houses.

A number of houses were subsequently cased with soil treated with chloropicrin in the same manner. In all cases a good control of the bubble disease was effected. It proved unnecessary to build a bin for each pile; instead, a binful was treated, then broken down after 48 hours, and moved over and assembled for the next lot. The end of the bin into which the soil is thrown may be built up with 1-ft. wooden boards as the soil is put in or it may be left entirely open. As Godfrey has pointed out, the water seal is unnecessary when the gas-impervious paper is used. As a matter of fact,

[2] The experiments were carried out in the houses of J. B. Swayne, Kennett Square, Pa.

soil treated in bins may simply be covered with burlap, newspapers, or building paper and then wetted down.

Production records were kept in two houses, one of which contained soil chloropicrin-treated, the other, soil treated with formaldehyde. The number of 10-lb. baskets taken out of each house during the 3 months of production was recorded. The soil treated with chloropicrin yielded 1.7 lb. per sq. ft. The formaldehyde-treated soil yielded 1.4 lb. per sq. ft. The difference cannot be attributed to the treatments. No replications were made. The results, however, show that chloropicrin does not impair the productive capacity of the soil. Previous observations tended to establish this point.

Increased fertility of the soil, sometimes noticed as a result of partial sterilization with chloropicrin, has not been obvious in these studies. One reason for this may be that the soil is not a source of nutrients for the mushroom. In mushroom culture the sole object of soil sterilization is the elimination of harmful organisms. If this be achieved without damaging the soil, the treatment may be regarded as satisfactory.

An attempt was made to treat soil in loose piles, but was promptly abandoned because it was difficult to obtain uniform distribution of the chloropicrin in loosely shifting soil. One Kennett Square grower has succeeded in controlling the disease by treating the soil in loose piles. This practice is, however, rather uncertain and is not at all recommendable.

In the preliminary experiments it was shown that spores of *Mycogone* were killed by a dosage of 2 ml. per cu. ft. Five ml. per cu. ft. were used in the bins in order to insure a lethal concentration under the variable conditions that might exist in the field. Experience with the bins indicated that confinement of the gas was good enough to permit a reduction in dosage. The soil for several houses was treated at the rate of 3 ml. per cu. ft. Observations on these houses indicated clearly that 3 ml. per cu. ft. were adequate.

FIELD TESTS

Fumigation of soil in bins is somewhat troublesome because the soil must be treated in 1 ft. layers. This labor is not prohibitive, since the soil must be piled on the composting ground, anyway. It would be simpler still, however, to inject the soil directly in the field prior to screening. Ordinarily, the soil is disked and then screened. It is thus in loose condition and suitable for treatment. Partial sterilization of the soil directly in the field has been satisfactorily accomplished for other purposes (2). A preliminary experiment was conducted to test this point. Two plots, 6 feet square, were disked to a depth of 8 inches and inoculated with spores of *Mycogone*. A ditch 1 foot wide and a foot deep was dug around each plot. Many lumps and clods of soil were lying freely on the surface, but no attempt was made to crush them, since the grower would not find it practical to do this. After infestation, the soil in each plot was mixed by turning with a shovel. From one of the plots about $\frac{1}{2}$ cu. ft. of soil was removed for subsequent use as a check. The plots were then marked out into 1-foot squares, and the

center of each square injected with 3 ml. of chloropicrin to a depth of 3 inches. Because of cost a heavier injection was not applied. Most growers use no more than the top 6 or 8 inches of soil. Both plots were sprinkled with water to create a water seal. One plot was covered with canvas and the edges buried all around in soil. The other was left uncovered. By the next day the surface of the uncovered plot had dried out. After 3 days the canvas was removed. The gas was distinctly detectable at that time. None could be detected in the uncovered plot. The daily afternoon temperature during this experiment was about 80° F. The soil of both plots was again thoroughly mixed. Enough was removed from each plot to case two trays similar to those employed in the first experiments. An added tray was cased with soil removed from one of the infested plots prior to treatment.

The trays were put into an empty house. The mushrooms on the non-treated soil began to emerge as typical bubbles and the tray was discarded. The soil from the treated, uncovered plot yielded mushrooms in both trays, which were about 35 per cent infected. These, too, were discarded. The soil from the plot covered with canvas after the treatment yielded disease-free mushrooms for 2 breaks, when they, likewise, were discarded.

It is obvious that adequate confinement of the gas is impossible with a water seal. This is true particularly during hot weather, when the water tends to evaporate readily and the gas volatilizes more quickly. On the other hand, the water seal and canvas covering were effective. Undoubtedly, the former can be eliminated. Godfrey has reported the water seal entirely unnecessary when the gas-impervious glue-coated paper is used. He estimates that this paper would cost not more than 2¢ per sq. ft. and it may be used repeatedly (3). The only objection to the use of strips of glue-coated paper is that they are difficult to keep in place on a windy day. This actually was the experience when they were tried in an experiment. On the other hand, canvas is heavier and may be had in large pieces. Whatever type of cover is used, a tight seal should be made around the edges. Three ml. per square foot suffices if the confinement of the gas is good. The importance of good confinement cannot be over-emphasized. No attempt was made to determine the depth of penetration of the gas, though it seems clear that it penetrated the top 8 inches.

Field treatment is a comparatively simple matter. Enough soil can be treated and covered in an hour to case a standard house. This amount of soil is contained in a plot about 25 ft. square if the top 6 inches are removed. It requires about 7 lb. of chloropicrin to treat this area in the field at the rate of 3 ml. per sq. ft. Half this amount is required for an equal amount of soil in the bins. It must be emphasized that the efficacy of the field treatment will depend on the degree of confinement of the gas. The surface of the field should be leveled off with a rake before the injections are begun. Not all the soil need be treated at one time. A plot may be treated one day and covered. After 48 hours, the cover may be transferred to the next

plot. In this way a relatively small amount of cover can be successively used for a large area.

The soil should not be allowed to remain in the field very long after the cover is removed because of danger of recontamination. It is inadvisable to treat the soil too far in advance of its use. Ordinarily, injections may be begun when the houses are filled. A week later the soil may be removed and piled on the composting ground, as usual.

FUMIGATION OF EMPTY HOUSES

Several trials were made to determine the value of chloropicrin as a fumigant. Formaldehyde or sulphur-dioxide ordinarily are used for fumigation of mushroom houses between crops. It was observed that chloropicrin did not evaporate rapidly enough when poured into a shallow pan. Rapid evaporation did occur when the chloropicrin was slowly poured along the length of the elevated walkway in the middle aisle. A gas mask was used during this operation. The fungicide was applied at the respective rates of $\frac{1}{8}$, $\frac{1}{4}$, and $\frac{1}{2}$ lb. per cu. ft. Spores of *Mycogone*, *Dactylium*, and *Trichoderma* were not killed by any of the fumigations. It was not considered advisable to use more than a half pound per thousand cubic feet because of the prohibitiveness of the cost.

CONCLUSION

Sterilization of casing soil with chloropicrin is a practical procedure that can be easily adapted to cultural practice. The fungicidal powers of chloropicrin cannot be doubted.[3] As a matter of fact, the field treatment with chloropicrin is as simple as sprinkling with Formalin and requires little effort or time. The grower may experience a little trouble with the special injectors at first until he learns the technique. It is essential to secure an adequate confinement of the gas. This may be easily managed by the use of a canvas. Merely wetting the surface of the soil after the treatment will not prevent rapid escape of the gas, especially in warm weather. Bin treatment is somewhat more troublesome than field treatment, but it is not at all prohibitively so. It is true, however, that only half the amount of chloropicrin used in the field is necessary when the soil is treated in bins. Soil should not be treated when the outside temperature drops below 60° F. Wet soils must be avoided because of the very limited solubility of chloropicrin. A loose, moist soil is ideal. A gas mask is not needed when the soil is treated outdoors. The treatment can be carried out with almost no inconvenience to the operator. Soil for the winter refill must be treated during warm weather and stored in a clean place. It is entirely practicable to inject the chloropicrin into the soil as it is being piled up in the storage shed; the technique is very simple and requires but little extra

[3] C. A. Thomas of the Pennsylvania State College has investigated the susceptibility of several species of mushroom house pests to chloropicrin. His studies were made coincidently with those reported in this paper and were carried out under the same conditions. The results of this work will be reported in the Journal of Economic Entomology.

effort. Since the stored soil will not be needed for 3 to 5 months, there will be a sufficient interval to permit the escape of the gas. A gas mask should be used if the storage shed does not admit a good supply of fresh air.

Actually, chloropicrin need replace formaldehyde only when it is suspected that mites or springtails are getting into the houses with the casing soil. One grower in North Carolina suffered material losses as a result of the damage caused by the long-legged mite, *Linopodes antennaepes*. He was able to eliminate the mite by fumigating his casing soil with chloropicrin. In instances of this kind, chloropicrin is, of course, more desirable than formaldehyde because of its insecticidal powers. At the present time Formalin is becoming somewhat difficult to obtain because of war priorities. Under these circumstances growers should not hesitate to use chloropicrin.

SUMMARY

Spores of *Mycogone*, *Verticillium*, and *Dactylium* were killed by chloropicrin used at the rate of 2 ml. per cu. ft. of soil. A method of treating soil in roughly constructed wooden bins is described. Three ml. of chloropicrin per cu. ft. gave good results in the bins. A method of treating soil directly in the field is described. In this method 3 ml. of chloropicrin are used for each square foot of surface. Good results are obtainable in the field if the gas be confined by an impervious cover. Soil treated with chloropicrin gave results comparable with those from soil treated with formaldehyde and steam. It is not economical to use chloropicrin for the fumigation of empty houses.

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A STRAIN OF TOBACCO-MOSAIC VIRUS CAUSING A NECROSIS AND SHRIVELING OF TOMATO FOLIAGE

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INTRODUCTION

At times during the past 6 years, greenhouse tomatoes at Beltsville, Maryland, have been affected by a mosaic disease causing necrosis and shriveling of the foliage. The disease has been observed only once in the field, but is known to have occurred as early as 1930 in greenhouses in Washington, D. C., and at Arlington, Virginia. On tomatoes it causes considerably greater injury than either the common green form of tomato (tobacco) mosaic (*Marmor tabaci* var. *vulgare* Holmes) or the yellow strain of the same virus (*Marmor tabaci* var. *aucuba* Holmes). It has been of particular interest because of its sporadic appearance in the greenhouse and the lack of evident sources of primary infection.

SYMPTOMS

Tomato

On tomatoes, the first symptom consists of a mottling much like that of the ordinary green tobacco mosaic, although of a slightly yellower type in young plants. This, however, does not resemble the bright, yellow mottle of the yellow or aucuba strain of tobacco mosaic. Within 12 to 15 days after inoculation, the older leaves begin to develop small, diffuse yellow areas, which show a minute, reddish-brown, necrotic stippling that gives these spots a characteristic russet-orange color (Fig. 1, B). These rusty patches gradually develop into large, pale-brown, papery spots that generally extend to the margins of the leaflets (Fig. 1, A). When so affected the leaflets are curled downward at the margins, twisted, and somewhat malformed (Fig. 2, A). Occasionally, an entire leaflet yellows rapidly, and fine, necrotic markings occur along the small veins (Fig. 1, C). This general yellowing of the older leaflets has been noted only in plants from 12 to 18 inches tall at the time of inoculation. It has occurred only when unusually low temperatures (65° to 70° F.) prevailed for 3 to 4 days just preceding the time when symptoms were first appearing.

As the disease progresses, the older leaves wither but remain attached to the plant. The withering of the foliage continues slowly up the stem and is readily confused with damage from fumigation or other chemical treatments (Fig. 2, B). There is no stem or petiole necrosis, and the new leaves show a green mottle with occasional reddish-brown necrotic areas that enlarge slowly. The affected leaflets are somewhat dwarfed and much curled and twisted. After they mature, these leaves gradually wither, but a fair amount of foliage always remains green at the upper portion of the plant. Only a few fruits are produced, but these are of normal appearance.

Leaf necrosis develops most rapidly at low temperatures. When held at temperatures ranging from 60° to 75° F., young, rapidly growing plants of 7 to 8 leaves have occasionally developed a general necrosis within 14 days after inoculation. The virus is readily transmitted by the ordinary leaf-rubbing technique or by brushing, handling, or pruning diseased and healthy plants.

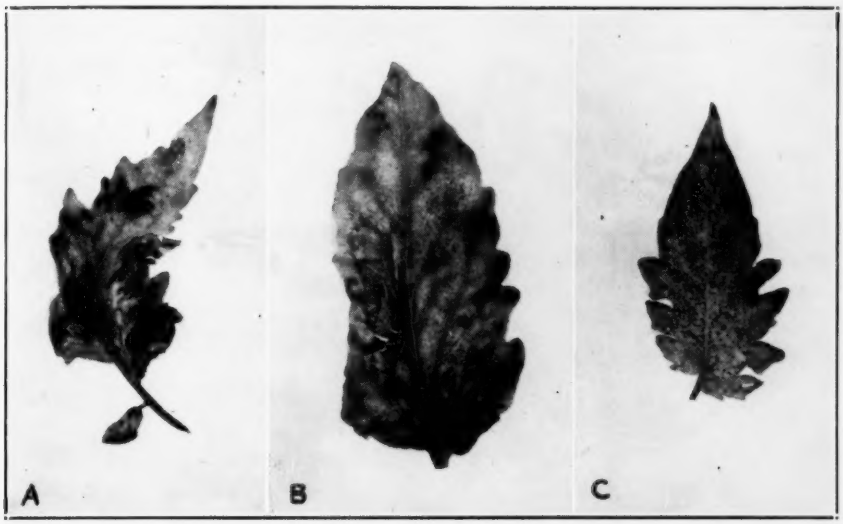

FIG. 1. Symptoms produced on tomato leaflets by the leaf-shriveling strain of the tobacco-mosaic virus. A, Typically curled and twisted leaflet showing yellowing and necrotic spots near the margin; B, leaflet showing necrotic spots and the russeting that precedes severe necrosis; C, leaflet entirely yellowed and covered with a fine, stippled necrosis.

Other Host Plants

As far as determined, the host range of the leaf-shriveling virus has been the same as that of tobacco mosaic. When tested in parallel series of inoculations on various wild species of *Lycopersicon*, the symptoms have been the same on all susceptible species. Those species showing a complete suppression of symptoms, when infected with the tobacco mosaic virus, have shown the same reaction to the tomato virus. The following species have been tested: *Lycopersicon pimpinellifolium* (Jusl.) Mill., *L. hirsutum* Humb. and Bonpl., *L. hirsutum* f. *glabratum* C. H. Mull., *L. peruvianum* (L.) Mill., *L. peruvianum* var. *dentatum* Dun. and *L. glandulosum* C. H. Mull.

On the Samsun variety of turkish tobacco (*Nicotiana tabacum* L.) the symptoms have always been the same as those of ordinary tobacco mosaic. At times, the tomato virus appears to produce a slightly yellower green of the entire leaf, but the pattern of the mottling is that of ordinary tobacco mosaic; no consistent differences in symptoms have been observed. There is no necrosis of leaf or stem and no yellow mottling of the aucuba type at any stage of growth.

On *Nicotiana rustica* L., *N. paniculata* L., and *N. quadrivalvis* Pursh.,

the symptoms also are like those of tobacco mosaic. The same is true of *N. glutinosa* L., where primary lesions are produced like those of tobacco mosaic, and the virus generally remains localized in the inoculated leaves. On *N. glauca* Graham, it differs from ordinary tobacco mosaic in the production of minute, superficial, reddish-brown, stippled lesions on the inoculated leaves but there is no systemic necrosis or mottling, although the virus becomes systemic.

FIG. 2. A, Tomato leaf showing curling, twisting, and necrosis of the leaflets typical of the leaf-shriveling virus; B, tomato plant showing shriveling of the older foliage.

On *Nicotiana sylvestris* Speg. and Comes, the tomato virus behaves like the aucuba type of mosaic in producing dark-brown, circular, necrotic primary lesions. It has not become systemic on this host except in the case of occasional young plants grown at high summer temperatures (90°–95° F.) in the greenhouse. In these plants there was a veinal necrosis and eventual death of all but the youngest leaves.

On pepper (*Capsicum frutescens* L.), *Physalis angulata* L., *Physalis*

pubescens L., *Physalis heterophylla* Nees., and *Physalis alkekengi* L. the symptoms are like those of tobacco mosaic and the same is true for *Datura stramonium* L., where the tomato virus produces primary local lesions but does not become systemic. On Scotia bean (*Phaseolus vulgaris* L.) the primary lesions also are like those of tobacco mosaic.

No infection has occurred in cucumber (*Cucumis sativus* L.), muskmelon (*C. melo* L.), watermelon (*Citrullus vulgaris* Schrad.), beet (*Beta vulgaris* L.), lettuce (*Lactuca sativa* L.), celery (*Apium graveolens* L.), or pokeweed (*Phytolacca decandra* L.).

RELATIONSHIP TO THE VIRUS OF TOBACCO MOSAIC

Physical Properties

As far as determined, the physical properties of the leaf-shriveling virus are the same as those of ordinary tobacco mosaic. A number of comparative determinations on young plants of tomato, turkish tobacco, and *Nicotiana glutinosa* have shown that the thermal destruction point of both viruses lies between 90° and 95° C. These trials were made with 2 cc. samples of the plant juices diluted 1 to 5 with tap water and heated for 10 minutes in a water bath controlled within $\pm$ 0.2° C.

Both viruses have produced a few lesions on *Nicotiana glutinosa* when diluted 1 to 1,000,000, and consistent infection has occurred on both turkish tobacco and *N. glutinosa* at dilutions of 1 to 100,000. Both viruses have been recovered in a rather high concentration from leaves of tobacco dried for 7 years at room temperatures, and also have remained active when held *in vitro* at room temperatures (21° to 27° C.) for 3 years.

Protective Reaction

The physical properties, together with the identity of host range and close similarity of symptoms on most of the host plants tested, indicate that the leaf-shriveling virus is a strain of *Marmor tabaci*. Further evidence on this point has been obtained from protective tests on *Nicotiana sylvestris* of the type described by Kunkel[1] in connection with studies of the aucuba strain of tobacco mosaic.

The leaf-shriveling virus, like that of aucuba mosaic, produces necrotic primary lesions on *Nicotiana sylvestris*, but is not ordinarily systemic. However, when plants of *N. sylvestris* are first inoculated with the tobacco-mosaic virus, and inoculations are then made with the tomato virus to leaves that begin to show symptoms of tobacco-mosaic infection, either no primary lesions are produced by the tomato virus, or such lesions as do occur are few in number and are apparently confined to areas of the leaf where the tobacco virus has not become fully systemic. Such protective action of the tobacco-mosaic virus further indicates that the leaf-shriveling virus is a related strain.

[1] Kunkel, L. O. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopath. 24: 437–456. 1934.

When tomato plants have been infected with the tobacco-mosaic virus for 30 days or more before inoculation with the tomato virus, there frequently is no later development of necrotic lesions or, at most, only a slight evidence of yellowing or necrosis. In tomatoes the degree of dominance of the green mottle of tobacco mosaic depends on the length of time the plants have been infected with this virus before subsequent inoculation with the leaf-shriveling virus. When tomato plants are inoculated with the latter virus within 15 days of the time the first symptoms of tobacco mosaic appear, they later develop a considerable amount of leaf necrosis. Plants first infected with the tomato virus, and then inoculated with the tobacco-mosaic virus after necrotic symptoms have appeared, show little reduction in the necrotic symptoms during later growth.

When tobacco plants are inoculated first with the tomato virus and later with tobacco mosaic (or *vice versa*) the symptoms remain those of tobacco mosaic. In all such plants the tomato virus persists in a fairly high concentration and produces abundant lesions in *Nicotiana sylvestris*. This also is true in all tomato plants, regardless of the degree of suppression of the necrotic symptoms by the tobacco-mosaic virus.

Serological Relationship

Additional evidence regarding this relationship to tobacco mosaic has been supplied by serological tests made through the kindness of F. O. Holmes and L. M. Black of the Department of Animal and Plant Pathology of the Rockefeller Institute for Medical Research. These tests have indicated that the tomato virus is serologically related to that of typical tobacco mosaic, since juices of tobacco plants infected with the tomato virus gave a characteristic precipitate when mixed with anti-serum of the tobacco virus. These tests have not necessarily eliminated the possibility that the plants carried tobacco mosaic plus another virus, but this seems unlikely in view of studies made with regard to such a possibility.

During the fall of 1940, some tomato plants inoculated with tomato virus developed numerous, small, circular spots on the inoculated leaves and on those directly above them. These spots were sharply defined and enlarged more slowly than did the diffuse, yellow areas characteristic of the virus. This suggested that we might be dealing with a combination of the ordinary green tobacco mosaic and a peculiar yellow strain. To test this, 52 inoculations were made to turkish tobacco, and 60 to Globe tomato, with fragments of tissue cut from the yellow spots. All of the inoculated tobacco plants showed the typical mottle of ordinary green mosaic. All of the tomatoes developed typical leaf necrosis, but only 6 showed the peculiar yellow spots. Twenty inoculations from green tissue of the same leaflets from which yellow tissue was taken all produced typical necrotic symptoms in tomato with two of the plants showing spotting. The same number of inoculations on tobacco produced only typical green mottling.

Another set of inoculations to tomato with necrotic tissue from lesions on

leaves of *Nicotiana sylvestris* inoculated from a yellow-spotted leaf, produced typical necrosis in all of the 36 plants inoculated, but no spotting occurred. Twenty-eight tobacco plants, similarly inoculated, showed typical green mosaic.

Further subinoculations from yellow spots on the leaves of the tomato plants inoculated with spot tissue failed to reproduce the spotting, and several later subinoculations from these plants never again produced spotting. Subinoculations to tomato from 34 of the tobacco plants inoculated with tissue from yellow spots also produced typical leaf necrosis, but none of the peculiar yellow spots.

These results, together with other data and observations on the variation in symptoms produced by this virus strain, have led us to believe that the peculiar spotting resulted from environmental factors rather than from a yellow-spotting strain mixed with a green type. Had the yellow spots been the localized expression of a yellow virus, occurring in comparatively pure form, one would have expected that inoculations from this tissue would result in a decidedly more yellow type of symptom on tobacco and tomato. Further, the fact that no yellow mottle or flecking has ever been noted with this virus on tobacco supports the belief that the disease on tomatoes is caused by a single virus.

In view of all the evidence on the strain relationship between the leaf-shriveling virus of tomato and typical tobacco-mosaic virus, it appears that we are dealing with a strain of the latter virus whose severe effect on tomato foliage is its most distinctive character. Under the classification of Holmes[2] it is proposed that this virus be known as *Marmor tabaci* v. *siccans* var. nov.

Sources of Primary Infection

The question of the sources of primary infection has presented a peculiar problem in the case of the leaf-shriveling virus because of its sporadic appearance in a single greenhouse at times when no infection was known to exist on plants in other houses nor, as far as could be determined, out-of-doors.

The greenhouse in which the disease has appeared is used for tomato breeding and two crops are grown each season. The soil is usually sterilized with steam before each planting and it does not seem likely that the infection is attributable to the presence of the virus in plant debris in the soil. This is confirmed by the fact that when one crop has been severely damaged, the succeeding crop frequently has shown no evidence of the leaf-shriveling virus.

The disease has been observed only once in the field and then was found on only a few plants that apparently had been infected while growing as seedlings in the greenhouse. No wild hosts have ever been noted near the greenhouses, the location of which precludes any common occurrence of such

[2] Holmes, F. O. Handbook of phytopathogenic viruses. Burgess Publ. Co. (Minneapolis). 1939.

weeds. The lack of general field infection also indicates that wild hosts are not prevalent. Furthermore, the disease has appeared in mid-winter in a year when it has not been found on the fall crop. The presence of the disease on inoculated plants might account for its appearance elsewhere in the greenhouse range, but the disease often has occurred when no inoculated material was present. Also, those working in the houses where the breeding crop is grown have had little or no contact with tomatoes in other houses, and care has been taken that they wash their hands before handling the plants.

In several instances, the first infection from the leaf-shriveling virus has occurred on small plants within two weeks after they have been transplanted to the ground-beds. In these instances, no evidence of ordinary tobacco mosaic infection has occurred until considerably later, and an examination of the seedlings remaining after transplanting has shown no other evidence of virus infection, even when they have been held for some time. The appearance of the disease on small plants does not necessarily indicate that the virus is carried in the seed, but it often has been found that the infected plants were grown from seed of plants also affected with the leaf-shriveling virus, and this, together with the peculiarly sporadic occurrence of the disease on the small plants, has seemed to warrant a study of the seed as a source of primary infection.

Inoculations on turkish tobacco and *Nicotiana glutinosa* with sterile-water extracts obtained by soaking samples of tomato seed from which the infected plants were grown, as well as other lots of seed from plants known to be infected, have always shown a high virus concentration on the coats of seed from 1 to 4 years old. This is in line with results reported by the writers[3] in trials made with seed from plants infected with ordinary tobacco mosaic.

Planting trials have been made with seeds of various ages known to carry the leaf-shriveling virus on their surface. In most instances these trials were conducted in greenhouses well isolated from other tomato plantings. The plants were grown in sterilized soil, either in flats, or more commonly, in pots. The flats and pots also were sterilized before use.

In these planting experiments, the only evidence of seed transmission has occurred with seed planted within ten days after extraction. In one experiment (1938), 183 seedlings were grown from seed from infected tomato plants. Five of these seedlings showed mosaic symptoms in the first true leaves, and developed the necrosis typical of the leaf-shriveling virus. Two of 246 seedlings grown from the same seed, after 10 days' drying, also were similarly infected. Three of the 123 control plants showed cases of ordinary mosaic, all of which occurred some time after that on the seedlings from infected seed, and were not accompanied by necrotic symptoms. While this series was felt to be indicative of virus transmission in freshly extracted

[3] Doolittle, S. P., and F. S. Beecher. Seed transmission of tomato mosaic following the planting of freshly extracted seed. Phytopath. 27: 800–801. 1937.

seed, it was not considered conclusive, since the plants were not completely isolated from other tomato plantings.

In a later experiment (1940) tests were made in which freshly extracted seeds were grown in an isolated greenhouse at a time when there were few tomato plants in any of the houses. The seeds were planted singly in pots of sterilized soil. The planting was so arranged that pots with seed from virus-free plants were alternated in a checkerboard arrangement with those containing seed from infected fruits. The seedlings were not touched after they appeared, except that those showing virus infection were carefully removed as soon as they were noted.

In two such series a total of 5 out of 342 seedlings from seed of infected fruit developed symptoms in the first leaf, and later showed a typical necrosis. All of these seedlings were much stunted, and the necrotic symptoms developed much more slowly than in older plants inoculated mechanically.

Later trials were made with the remainder of the same seed after it had been dried for 8 days (49 plants), 20 days (51 plants) and 35 days (117 plants). The arrangement and location of the experiments were the same as in the previous trials. In these experiments all of the plants, including 196 controls, remained healthy.

In tests with 1,422 tomato plants grown from seed taken from infected fruits and aged for 30 days to 3 years, only one plant has shown evidence of the disease. This single infection was an apparent contamination, since it did not occur until after the plants had grown for over 30 days. All of the 617 controls, grown from seed of mosaic-free plants, remained healthy. Observations of over 3,000 seedlings, grown from seed of infected plants in the course of other experimental work, have indicated that, as with the ordinary tobacco-mosaic virus, aged seed is rarely if ever a source of primary infection.

These results are like those reported by the writers[4] in the case of the virus of ordinary tobacco mosaic. While this occurrence of the leaf-shriveling disease on seedlings grown from freshly extracted tomato seed is of interest, the results do not account for the greenhouse infections, since in those instances the tomato plants were grown from seed stored for 60 days to 1 year or more.

There is, perhaps, a possibility that the necrotic virus may occasionally occur in manufactured tobacco, since its effect on the tobacco plant is no more severe than that of ordinary mosaic, and would not prevent the use of the leaves for commercial purposes. This is possible, but, if so, the virus should be fairly common on tobacco, and, under such circumstances, one would expect it to occur more frequently on tomatoes in the field. At present it is difficult to account for its appearance in the greenhouse under the circumstances in which it has occurred.

[4] See footnote 3.

SUMMARY

Tomatoes in experimental greenhouses in the vicinity of Washington, D. C., at times have been affected by a virus causing a reddish-brown necrosis of the leaflets followed by a gradual shriveling of the older foliage. There are no symptoms on the stems or fruit.

As far as determined, the physical properties and host range of this virus are the same as those of ordinary tobacco mosaic, and the symptoms are the same on tobacco and on nearly all other hosts tested. Serological tests indicate a relationship between the two viruses.

On *Nicotiana sylvestris*, where the tomato virus produces lesions like those of aucuba mosaic, the previous infection of the plants by the tobacco mosaic virus protects against infection by the virus from tomato.

Since all comparative evidence indicates that the virus causing leaf-shriveling of tomato is a strain of the tobacco mosaic virus it has been classified as *Marmor tabaci* var. *siccans* var. nov.

Seed transmission has occurred in tomato seedlings grown from freshly extracted seed of infected fruits, but no such transmission has been noted when seed was dried for more than 10 days.

The virus has appeared sporadically in the greenhouse for several years, but the sources of primary infection remain in doubt.

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THE REACTION OF CANTALOUPE STRAINS TO POWDERY MILDEW

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INTRODUCTION

Cantaloupe powdery mildew (*Erysiphe cichoracearum* DC.) first assumed epiphytotic proportions in the Imperial Valley, California, in 1925 and 1926 (1, 4). As a result of investigations by the U. S. Department of Agriculture and the California Agricultural Experiment Station, a variety was developed by 1936 that seemed highly resistant to the parasite. This variety, Powdery Mildew Resistant Cantaloupe No. 45, proved very satisfactory from the standpoint of both the grower-shipper and the consumer (2). With the advent of No. 45 into general usage the disease problem seemed to have been solved, at least temporarily. In 1938, however, a new race of *E. cichoracearum* appeared to which No. 45 and all other commercial varieties of cantaloupe were extremely suspectible (3). Since then several supposedly tolerant melon selections have become available to growers, but it is not known how these strains will behave under very severe disease conditions.

Preliminary work has indicated that in our melon[1] seed stocks there are some strains highly resistant to both races of the pathogen, but the genes for resistance are scattered through a wide range of material. Furthermore, the material was heterozygous for resistance factors and the melons were not commercially acceptable. It is, therefore, necessary to collect the genes concerned in producing immunity and combine them with factors responsible for desirable commercial qualities in the vines and fruit.

Before satisfactory progress in developing an acceptable, resistant cantaloupe variety or, even before the so-called tolerant strains can be adequately checked for ability to withstand the ravages of mildew, a rapid and precise method of gauging individual plant resistance must be devised. If such a technique were available for greenhouse use, resistance could be determined and susceptible lots eliminated before planting in the field. Further, resistant plants in a segregating population could be detected and set out in the field for observation and use in the breeding program. Such a procedure would not only dispense with land used and time consumed in growing worthless susceptible plants but also would enhance the possibility of securing immune plants with good fruit quality.

Therefore, it has been the purpose of the present investigation (1), to devise a greenhouse method of testing for mildew resistance that would be more rapid and reliable than natural field infection and, with this technique (2), ascertain the range of resistance in the breeding material at hand,

[1] Terminology used by Wiant (10) is followed in this paper.

and (3), determine the mildew tolerance of varieties now available to commercial growers.

MATERIALS AND METHODS

In the greenhouse, plants were inoculated with a culture of race 2 of *Erysiphe cichoracearum*, described elsewhere (5), when the first true leaves had appeared. The inoculation technique and equipment used in other research (5) were employed. Disease notes were taken 16 days after inoculation. After the reaction of each plant had been noted, desirable individuals were transplanted to field plots at Torrey Pines, and Brawley, California, to determine their resistance under field conditions. Disease notes in field plantings were taken at about the time the first melons were ready for harvest.

Supposedly tolerant[2] strains were checked for resistance in the greenhouse and seed of the same lots planted in randomized and replicated field plots near Brawley in 1941. Plantings of 18 cantaloupe strains with 18 plants per strain in each of 3 blocks were made at 3 different dates. An analysis of variance (7) was made on these data.

EXPERIMENTAL RESULTS

Greenhouse Symptoms[3]

It is necessary to understand the manner in which plants, differing in ability to withstand mildew attack, respond to artificial inoculation with the fungus before the reaction of various melon strains can be adequately studied and before an arbitrary mildew-resistance rating can be devised. For these reasons a detailed description of powdery-mildew symptoms is given below:

Leaves. Macroscopically visible mycelium appeared on susceptible plants 4 to 6 days after inoculation and 7 to 10 days later mycelium covered the leaf. In the different cantaloupe strains the incubation period, mycelial development, and degree of sporulation varied, depending upon plant resistance (Fig. 1). Of these factors the incubation period seemed to be influenced least by the different host strains. The more resistant the plant, the longer the leaf remained green and vegetative.

Another type of reaction, which also seemed to be related to the resistance of the various melon strains, was a range of necrotic or chlorotic spotting, generally with mycelial development suppressed or absent (Fig. 1, C). Badly necrotic areas eventually became dry and brittle. When a great number of conidia were concentrated in a small area of a fully expanded resistant leaf during the inoculation procedure, severe necrosis often resulted in this spot.

Cotyledons. The symptoms on the cotyledons were similar to those on leaves, but in only a few plants of some strains did these structures remain entirely mildew-free. In general, they seemed more susceptible than leaves.

[2] Throughout this paper the expressions "tolerant," "susceptible," "resistant," and the like refer to host reaction to race 2 of the powdery mildew unless otherwise stated.

[3] A preliminary report of these symptoms has already been made (6).

Macroscopically visible mycelium appeared on cotyledons of susceptible plants 1 to 2 days earlier than on the leaves. Since they were exposed before the first true leaf unfolded, this earlier appearance of mildew may have resulted from inoculum from nearby infected plants. The rate of senes-

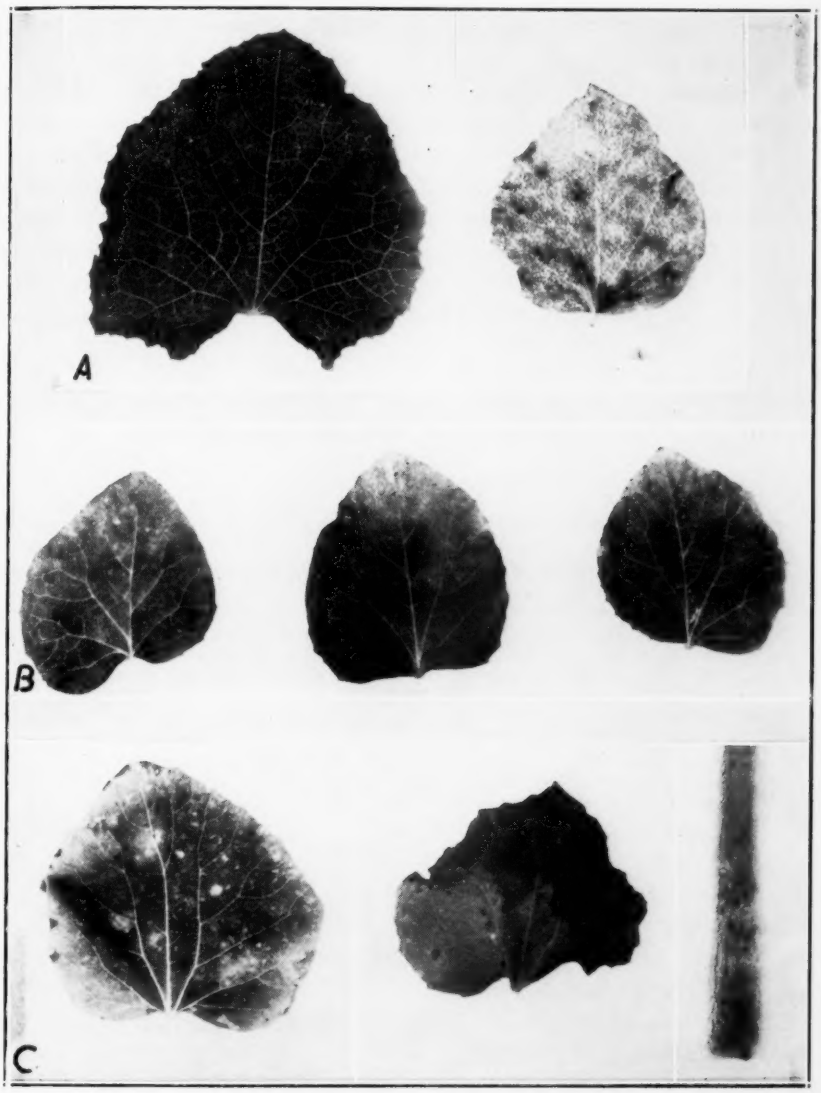

FIG. 1. Powdery mildew on cantaloupes differing in resistance. A. Highly resistant leaf compared with a susceptible leaf. B. Leaves upon which mycelial development and sporulation is suppressed. C. Leaf necrosis and stem cracking.

cence increased with the severity of infection; in many cases death resulting from senescence was difficult to distinguish from that caused by the fungus.

Stems. Visible mycelium usually appeared somewhat later on the stems

of susceptible plants than on the leaves. This may not have been attributable solely to a longer incubation period, but, rather, to a less severe or delayed inoculation. The stems being vertical and shaded by the leaves would have had less surface exposed to air-borne falling spores. The degree of stem reaction was related in general to the extent of mycelial development on the leaves, except for plants of some strains in which the leaves became infected, while the stems remained free, and certain others in which the leaves were free, while the stems were mildewed. In a few cases the organism spread from the basal portion of heavily attacked cotyledons so that it entirely encircled the stem, but the fungus did not develop further from this point. No symptom exactly similar to leaf necrosis was observed on the stems, but a type of longitudinal cracking was noticed occasionally (Fig. 1, C). Frequently, stem necrosis appeared at the soil line. Since many spores were washed from the leaves and cotyledons to the soil during the watering, corrosion at this point may have resulted in some cases from repeated inoculation on resistant stems.

Rating the Amount of Mildew. For convenience in taking notes and recording results symptoms have been designated as follows:

Type 0—No mycelium evident to the naked eye.

Type 1—Only 1 to 3 small colonies developed. These rarely enlarged to more than 2 or 3 mm. diameter; seldom sporulated abundantly. In a few cases it was noticed that on plants with this grade of resistance, mycelial colonies had elongated over midrib or main veins, or sometimes concentrated at base of midrib where it joins the petiole—very slight infection.

Type 2—Little mycelium developed and few conidia formed. Colonies few, 1 to 4 mm. in diameter; confined to no particular area or there was rather general development of very diffuse mycelium giving faint grayish tinge to infected areas—slight infection.

Type 3—Mycelium sparsely covered part or all of the leaf. Sporulation somewhat suppressed—medium infection.

Type 4—The mycelium entirely covered the leaf. Sporulation abundant—severe infection.

Type A—Used in connection with the other reaction types to indicate stem cracking and necrotic or chlorotic spotting on leaves and cotyledons. No attempt made to evaluate degree of necrosis, since in most strains the symptom was mild.

FIELD REACTION

For comparison with strains tolerant to race 2, No. 45 and a highly resistant inbred were employed. Most of these strains had been mass-increased in isolated plots and, consequently, were more or less heterozygous for fruit and vine characteristic and for mildew resistance. The relative reactions of these 18 strains are shown in table 1. A partial description of these strain is as follows:

1.—No. 45, susceptible to powdery mildew race 2.

2 to 8.—Selections from No. 45, thought to possess some mildew resistance.

9.—An F_5 selection from a cross of No. 45 with a cantaloupe of unknown origin. In the greenhouse this strain has always been free of macroscopically visible mycelium but responds to artificial inoculation with the production of necrotic spots. Although the fruit is not desirable commercially, it is used here as a standard for high resistance.

10.—Powdery Mildew Resistant No. 8. This strain was thought to possess some mildew tolerance; in certain crosses seems to have contributed factors for resistance.

11.—An F_4 selection from a cross between No. 45 and No. 8.

12 to 15.—Selections from commercial D–2. This type (D–2), apparently the result of a chance cross between No. 45 and No. 8.

16.—An F_3 selection from a cross between No. 45 and D–2.

17 and 18.—Selections from Hale's Best, a commercial variety, susceptible to both races of pathogen.

TABLE 1.—*Mildew reaction of some cantaloupe strains in field plots at Brawley, where they were exposed only to natural infection*

Melon strain[a]	Average disease index[b]		
	First planting	Second planting	Third[c] planting
1	3.778	4.000[c]	4.000
2	2.167	2.463	4.000
3	2.759	3.463	4.000
4	2.037	3.037	4.000
5	3.519	3.611	4.000
6	3.278	3.463	4.000
7	2.889	3.815	4.000
8	3.463	3.352	4.000
9	0.000[c]	0.000[c]	0.000
10	3.593	3.352	4.000
11	3.685	3.741	4.000
12	2.389	2.444	4.000
13	2.981	3.241	4.000
14	2.926	3.241	4.000
15	2.833	3.074	4.000
16	3.481	3.704	4.000
17	3.352	3.667	4.000
18	1.593	2.926	4.000
Least signif. mean diff. at 5% level	0.771	0.777	.....
Date of planting	12/4/40	12/23/40	1/27/41
Date of notes	5/8/41	5/21/41	6/11/41

[a] All strains except No. 9 rated 4.0 in the greenhouse test.

[b] Based upon amount of mycelium and degree of sporulation: 0—no mycelium; 1—very slight amount of mycelium; 2—slight mycelium, sporulation suppressed; 3—medium mycelium, sporulation suppressed somewhat; 4—abundant mycelium, sporulation vigorous.

[c] Not included in the statistical analysis.

Usually, early melons are more severely attacked by powdery mildew than are those of other plantings. The early-cantaloupe region is largely confined to the western part of the Imperial Valley, where the frost hazard is least and the soil is fairly light. This area also produces much early squash upon which some mildew can almost always be found throughout the winter months. The more severe infection of these early melon plantings may perhaps be attributed to the more or less constant presence of inoculum and to environment favorable for development of the parasitic complex. It will be noticed that the early plantings at Brawley (Table 1) had considerably less mildew, the difference between plantings being statistically significant. Since these plots were about 6 miles from early melons or squash, scarcity of inoculum may account in part for the lower disease incidence. There were significant differences between blocks in the second planting but they could not be accounted for on the basis of present knowledge.

In the first planting, 9 of the melon strains selected for powdery mildew

TABLE 2.—*Powdery mildew reaction of cantaloupe plants inoculated in the greenhouse and transplanted to the field where they were exposed to natural infection*

Transplanted to field plots at Torrey Pines							Transplanted to field plots at Brawley			
Greenhouse reaction[a]			Field reaction[a]				Greenhouse reaction[a]			Field reaction[a]
Plant organ	Mildew reaction	Number of plants	0	2	3	4	Plant organ	Mildew reaction	Number of plants	0
			No. plants	*No. plants*	*No. plants*	*No. plants*				*No. plants*
Leaf	0	49	46	1	2	0	Leaf	0	43	43
	0A	73	70	0	3	0		0A	27	27
	1	8	7	0	0	1		1	13	13
	1A	11	11	0	0	0		1A	7	7
	2	6	5	0	0	1		2	21	21
	2A	6	4	0	1	1		2A	4	4
	3	9	6	0	1	2		3	4	4
	4	4	2	0	0	2		.....	.....	.....
Stem	0	66	63	1	1	1	Stem	0	66	66
	0A	32	29	1	2	0		0A	9	9
	1	13	13	0	0	0		1	10	10
	1A	9	9	0	0	0		2	6	6
	2	14	12	0	1	1		3	22	22
	2A	5	5	0	0	0		4	11	11
	3	17	15	0	0	2		.....	.....	.....
	3A	2	2	0	0	0		.....	.....	.....
	4	8	4	0	3	1		.....	.....	.....

[a] Only those disease index groups in which plants occurred are included in the table. Disease index based on amount of mycelium, degree of sporulation, and presence or absence of necrosis or chlorosis: 0—no mycelium; 1—very slight amount of mycelium; 2—slight mycelium, sporulation usually suppressed; 3—medium mycelium, sporulation usually somewhat suppressed; 4—abundant mycelium, sporulation vigorous; A—presence of necrosis or chlorosis. Notes on necrosis were not taken in the field because the symptom was difficult to separate from that caused by agents other than powdery mildew.

tolerance (disregarding number 9) were significantly less susceptible than number 1, but none of them approached the high resistance found in number 9. Except for numbers 8, 9, and 10, the second planting was more severely attacked than the first. In this planting only 5 of the tolerant lines appeared to be significantly more resistant than number 1. In the last planting, all of the strains, except the highly resistant number 9 were very heavily infected.

Comparison of Greenhouse Inoculation and Natural Field Infection

All of the melon strains included in the field plantings (Table 1) have been repeatedly tested in the greenhouse, where all, except number 9, have developed a 4 reaction on the leaves, stems, and cotyledons within 16 days after inoculation. From these data it appears that certain mildew-tolerant strains may be comparatively disease-free when the inoculum is scant or environmental factors are unfavorable for the disease, but under epiphytotic conditions they are no better than commercial No. 45. Greenhouse tests lend support to this supposition.

A great many other strains were subjected to greenhouse inoculation. From these, promising individual plants were selected and transplanted to field plots at Torrey Pines or at Brawley, where observations of field resistance were made. Records for a representative number of these selected plants in the 1940 and 1941 plantings are shown in table 2. A large majority were completely free of mildew at the time the first melons were picked.

In a few cases leaves or stems of some plants appeared to be more severely infected in the field than in the greenhouse, indicating that the greenhouse method was not always so effective in producing severe disease symptoms as

TABLE 3.—*Powdery mildew reaction of individual cantaloupe plants that appeared to be more resistant in the greenhouse than in the field*

Plant number	Greenhouse reaction[a]			Field reaction	
	Leaves	Cotyledons	Stems	Leaves	Stems
8–2M–2	0A	4A	0	2	2
16046–1	0A	4	3	2	0
16111–1	0	3	0A	3	3
16111–2	0	4	0A	3	3
16111–3	0	4	0A	2	2
29082–3	1	4	2	4	3
29442–10	2	2	2	4	4
29442–12	3	4	4	4	3
29442–13	0A	0	0	2	3
29443–3	2A	4	3	4	4
29443–5	4	4	3	4	4
29443–6	3	3A	0	4	4
29443–14	3	3	4	3	3
29443–16	2A	3A	4	3	3

[a] Disease index based on amount of mycelium, degree of sporulation and presence or absence of necrosis or chlorosis: 0—no mycelium; 1—very slight amount of mycelium; 2—slight mycelium, sporulation usually suppressed; 3—medium mycelium, sporulation usually somewhat suppressed; 4—abundant mycelium, sporulation vigorous; A—presence of necrosis or chlorosis.

were natural field conditions. Of particular interest in this connection are plants that had a 0 reaction on leaves or stems in the greenhouse, yet showed some mildew in the field, since these individuals probably would be considered desirable as resistant parents. Table 3 shows the powdery mildew reaction of the individual plants whose leaves or stems appeared more resistant in the greenhouse than in the field. In the greenhouse all of these plants manifested symptoms of mildew either on the leaves or the stems, although not necessarily on both organs of the same plant. For example, plant 16111–1 showed a 0 reaction on the leaves, but a 0A reaction on the stems in the greenhouse, indicating a slight degree of infection; whereas the field reaction was 3 for both leaves and stems. However, at no time during the two years this problem has been under investigation has a plant with a 0 reaction on both leaves and stems in the greenhouse developed any mildew in the field. In only a few cases have plants, free of macroscopically visible mycelium on leaves or stems, but showing some necrosis or chlorosis, had mildew when transplanted to field plots. On the other hand, quite a few plants rated 1, 2, or 3 in the greenhouse have become more severely attacked in the field.

From the above data it is evident that (1) there exists in our breeding stock strains that are highly resistant to powdery mildew, both in the greenhouse and in the field; (2) the greenhouse technique may be used effectively in selecting very highly resistant plants; (3) it is not so reliable in evaluating different degrees of field tolerance; (4) the reaction of both leaves and stems must be considered in making selections.

DISCUSSION

Repeated tests have illustrated that a uniform and severe disease infection always may be obtained in the greenhouse, whereas, in the field, host reaction to mildew is not the same throughout the year. Summer and fall plantings in the Imperial Valley often are made to increase seed of desirable lines. Observations over several years have shown that these plantings almost always remain mildew-free. Environmental factors may account for this lack of infection. Yarwood (11) suggested that high temperatures, combined with low humidity, may be injurious to the powdery mildews. His studies were made in part on *Erysiphe cichoracearum* from sunflower, and may not be exactly comparable to the biologic race on cantaloupe. However, the field observations mentioned above indicate that the cantaloupe race of the pathogen may be sensitive to high temperature and low humidity, which, if true, would reduce inoculum during the summer. Light intensity and plant resistance at different ages also should not be excluded from consideration as factors possibly influencing the amount of mildew.

In the early plantings, where mildew is often severe, environment may possibly tend to reduce the resistance of lines that at other times of the year are comparatively disease-free. Although the effect is reversed, a situation perhaps comparable is found in the "type B" resistance of cabbage to yel-

lows (8, 9); there, high temperature brings about disease development in certain varieties that, during cool weather, seldom show severe yellows symptoms.

Plants with some necrosis or a small amount of mycelium on leaves or stems under greenhouse conditions cannot always be relied upon to be highly resistant in the field. However, many strains, having leaves with necrotic spots but no mycelium visible to the naked eye, did not become infected in the field plantings. From the results of this investigation it would seem that the greenhouse method of eliminating susceptible lines is more efficient and reliable than field trials performed throughout the year, provided all macroscopic leaf and stem symptoms be taken into account.

SUMMARY

Cantaloupe plants, differing in resistance to powdery mildew, were artificially inoculated in the greenhouse with race 2 of *Erysiphe cichoracearum*, and the range of symptoms described. Five classes of symptoms based on mycelial development and degree of sporulation were employed in rating the susceptibility of these plants, 0 to 4 indicating the range from absence of visible mildew growth to abundant, vigorously sporulating mycelium. Leaves and cotyledons of some plants also responded with the production of faint yellow to definitely necrotic spots, usually with mycelium restricted or absent. No symptoms exactly similar to leaf or cotyledon spotting appeared on stems but a longitudinal cracking was noticed occasionally. Leaf, cotyledon, and stem reaction differed greatly between the various melon strains; and, on individual plants, these organs were seldom attacked with equal severity, except in the susceptible strains, where they were entirely covered with mildew. Other conditions being constant, the symptoms produced on leaves, cotyledons, or stems appeared to be a function of the host genotype. In general, cotyledons seemed more susceptible than leaves or stems.

Duplicate plantings of 18 cantaloupe strains were made in the greenhouse and at 3 different dates in field plots. These 18 lines comprised selections from Hale's Best (susceptible to both mildew races), Powdery Mildew Resistant Cantaloupe No. 45 (resistant to race 1 but not to race 2), a strain highly resistant to both races of the pathogen, and several strains thought to be tolerant to both mildew forms. In the greenhouse all strains, except the one highly resistant to both fungus races, had a 4 reaction on leaves, stems and cotyledons. Mildew severity increased in each successive field planting, all the plants in the third planting, except the highly resistant strain, having a 4 reaction. The majority of the tolerant strains were significantly better than No. 45 in the first planting, and several were superior in the second.

Data from the reaction of individual plants in the greenhouse and of these same plants after being set out in the field showed that the greenhouse method is much more reliable than field trials as a tool for selecting highly

resistant plants, provided all macroscopic leaf and stem symptoms are taken into account.

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CROWN GALL ON SPECIES OF TAXACEAE, TAXODIACEAE, AND PINACEAE, AS DETERMINED BY ARTIFICIAL INOCULATIONS[1]

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Results of inoculation of species of Pinaceae and Taxaceae with *Phytomonas tumefaciens* (Smith and Townsend) Bergey *et al.* have been reported by the writer on *Sequoia* spp. (1), on *Araucaria bidwillii* (2), *Libocedrus decurrens* (3), *Taxus baccata* var. *erecta* (4), and on species of Cupressaceae (5). Results of further studies are herein reported.

The trees for these tests were secured from local nurseries; they were established in 5-gal. containers and grown in a lath house. Trees of *Sequoia* spp. also were grown in the open.

Inoculations were made by the puncture method, with a sterilized steel needle and pure cultures of the crown-gall organism. Control punctures were made on all the different species under experimentation; and in all cases the wounds healed normally.

TABLE 1.—*Summary of inoculations with Phytomonas tumefaciens on species of conifers*

Species inoculated	Number of inoculations	Number of galls	Diameter range (mm.)
Taxaceae			
Cephalotaxus fortunei	75	0	.....
Podocarpus elongata	145	3	15–22
Podocarpus macrophylla	15	0	.....
Taxus baccata var. *erecta*	80	50	10–20
Taxus brevifolia	15	4	5–15
Taxus cuspidata	65	0	.....
Taxus media	45	4	2– 4
Torreya californica	15	7	10–30
Taxodiaceae			
Cryptomeria japonica	60	0	.....
Cryptomeria japonica var. *elegans*	70	0	.....
Cunninghamia lanceolata	40	9	6–40
Sciadopitys verticillata	65	5	2– 5
Sequoia gigantea	35	6	15–25
Sequoia sempervirens	60	4	2–60
Taxodium distichum	50	0	.....
Taxodium mucronatum	220	0	.....
Pinaceae			
Abies cephalonica	66	2	5–10
Abies concolor	37	5	5–20
Abies firma	81	1	10
Abies holophylla	21	2	5–25
Abies nephrolepis	15	0	.....

[1] Paper No. 462, University of California Citrus Experiment Station, Riverside, California.

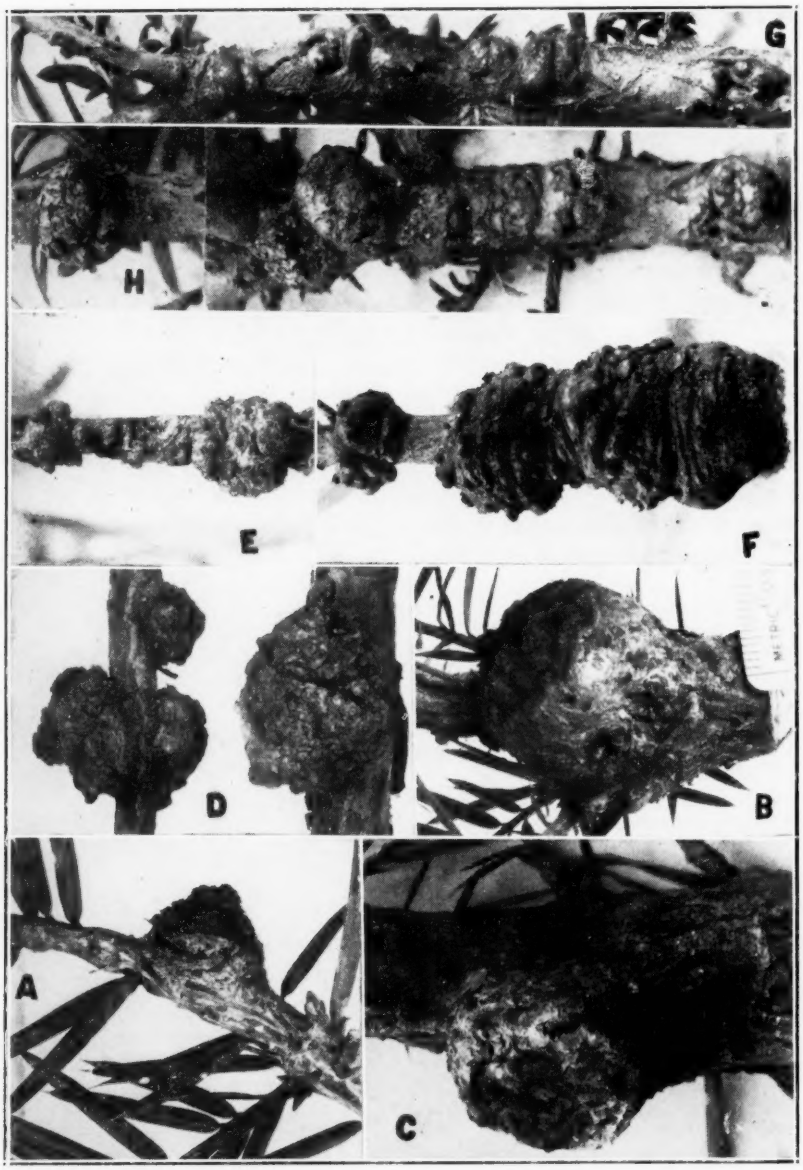

FIG. 1. Artificial galls produced on species of Taxaceae by inoculation with *Phytomonas tumefaciens* isolated from *Salix* sp. for all galls except those on *Taxus baccata* var. *erecta*, for which cultures were isolated from *Prunus persica*. A to C. Galls on *Torreya californica:* A, 15 months after inoculation; B, after 22 months; C, after 29 months. D. Galls on *Taxus baccata* var. *erecta*, after 60 months (galls long inactive). E and F. Galls on *Podocarpus elongata:* E, 11 months after inoculation, showing smooth, spherical form of early stages; F, after 40 months, showing irregular form and small roundish projections of later development. G and H. Galls on *Taxus brevifolia:* G, 30 months after inoculation; H, after 45 months; note increase in size of certain galls but slight change or no change in others.

INOCULATION OF SPECIES OF TAXACEAE

Species of Taxaceae tested for crown gall by artificial inoculations were as follows: *Cephalotaxus fortunei* Hook., *Podocarpus elongata* L'Her., *P. macrophylla* Don, *Taxus baccata* var. *erecta* Loud., *T. brevifolia* Nutt., *T. cuspidata* Sieb. and Zucc., *T. media* Rehd., and *Torreya californica* Torr. The small trees of these species were inoculated at frequent intervals during the year. The cultures used on *Taxus baccata* var. *erecta* were from *Prunus persica* L. All other cultures were from *Salix* sp. Results of the inoculations are summarized in table 1.

In these tests, the artificially induced galls (Fig. 1) were not always entirely typical of crown gall as it appears on other hosts. Inoculation of *Podocarpus elongata* caused typical spherical overgrowths in the early stages of development (Fig. 1, E); but in later stages these growths became more irregular in form, and small roundish projections developed (Fig. 1, F). The results presented in table 1 suggest that *P. elongata* is strongly resistant.

Torreya californica was readily infected. The galls (Fig. 1, A to C) were slow in appearing, but, once started, their development was continuous and fairly rapid. In their early stages, some of the overgrowths were smooth (Fig. 1, B) and some were rough (Fig. 1, A). In later stages, however, galls on this host generally became rough, much like the overgrowth shown in figure 1, C.

The galls on *Taxus brevifolia* (Fig. 1, G and H) and on *T. baccata* var. *erecta* (Fig. 1, D) grew slowly, but were more typical of crown galls than those on *Podocarpus*. Some of the galls on *T. brevifolia* are still growing; others are inactive and have made but slight growth in the 2 years since inoculation. This host has, itself, made but slight growth during this time. Galls on *T. baccata* reached their maximum size, and have long been desiccated and dead.

The results of the inoculations on *Taxus cuspidata* were inconclusive. Small galls appeared on *T. media*, however, which is a hybrid between *T. cuspidata* and *T. baccata*. All the tests on *Cephalotaxus fortunei* were negative. Results of previous tests on this species were also reported as negative (4). It appears that *C. fortunei* may be resistant.

INOCULATION OF SPECIES OF TAXODIACEAE

The following species of Taxodiaceae, an important family of the conifers, were inoculated in these tests: *Cryptomeria japonica* Don, *C. japonica* var. *elegans* Masters, *Cunninghamia lanceolata* Hook., *Sciadopitys verticillata* Sieb. and Zucc., *Sequoia gigantea* DC., *S. sempervirens* Endl., *Taxodium distichum* Rich., and *T. mucronatum* Ten. Results of the inoculations are summarized in table 1.

Cryptomeria japonica, *C. japonica* var. *elegans*, *Taxodium distichum*, and *T. mucronatum* did not respond to inoculation. Galls were readily produced on *Cunninghamia lanceolata* (Fig. 2, D), *Sequoia gigantea* (Fig. 2,

C), *S. sempervirens* (Fig. 2, A and B), and on *Sciadopitys verticillata*. The galls were especially well developed on *Cunninghamia lanceolata*, and this species was apparently extremely susceptible. The susceptibility of *Sequoia* spp. to crown gall was previously reported (1) and is confirmed by the results of inoculations in these tests. The galls on *Sciadopitys* were small but typical. This host, under conditions at Riverside, California, made slow growth, and the resultant galls were small knobs 2 to 3 mm. in diameter.

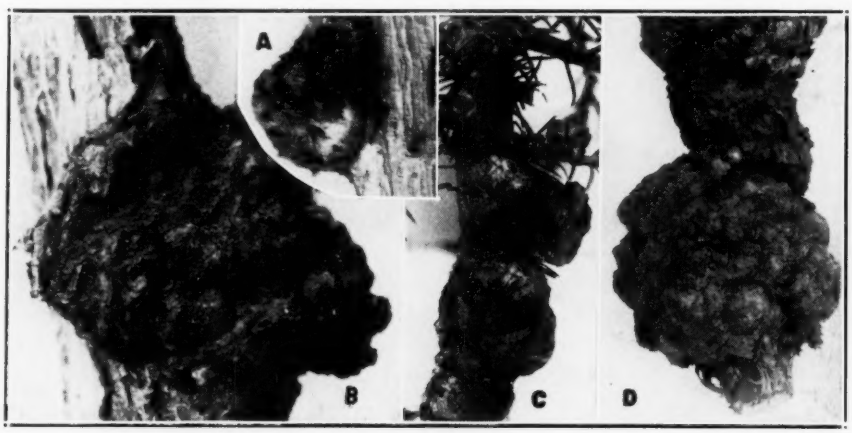

FIG. 2. Artificial galls produced on species of Taxodiaceae by inoculation with the crown-gall organism isolated from *Salix* sp. A and B. Gall on *Sequoia sempervirens:* A, 24 months after inoculation; B, after 30 months. C. Three galls on *S. gigantea*, after 7 months. D. Galls on *Cunninghamia lanceolata*, after 33 months.

INOCULATION OF SPECIES OF PINACEAE

The Pinaceae tested for crown gall by artificial inoculations included species of *Pinus*, *Picea*, *Pseudotsuga*, *Cedrus*, *Larix*, and *Abies*. No galls were obtained on the following species of *Pinus: P. canariensis* C. Smith, *P. cembroides* Zucc., *P. coulteri* Don, *P. halepensis* Mill., *P. muricata* Don, *P. pinea* L., and *P. sylvestris* L. Results on *Picea koyamai* Shiras (SPI 97948), *P. pungens* Engelm., *Pseudotsuga taxifolia* Brit., *Ps. macrocarpa* Mayr., *Cedrus deodara* Loud., *Larix decidua* Mill. (*L. europaea* DC.), and on *L. kaempferi* Sarg. (*L. leptolepis* Murr.) also were negative.

Those species of *Abies* tested showed, with the exception of *A. nephrolepis* Maxim. a definite response to artificial inoculations (Fig. 3). Inoculations were necessarily made on wood several years old. The growth of these hosts was slow, and conditions were thus unfavorable for gall formation. But, though few galls were produced from a relatively large number of inoculations, these galls were typical. Results of the tests are given in table 1.

The gall on *Abies concolor* Lindl. and Gord. (Fig. 3, E) showed old tissue still attached but being pushed off by the formation of new gall tissue. The galls (Fig. 3, C and D) were well developed and more typical. Galls on *A. holophylla* Maxim. (Fig. 3, A and B) were globose and apparently at-

tached to the host by a small area of tissue. The galls produced on *A. firma* Sieb. and Zucc. (Fig. 3, F) and on *A. cephalonica* Loud. were on trees that were kept under a moist chamber in the lathhouse during and for some time after inoculation.

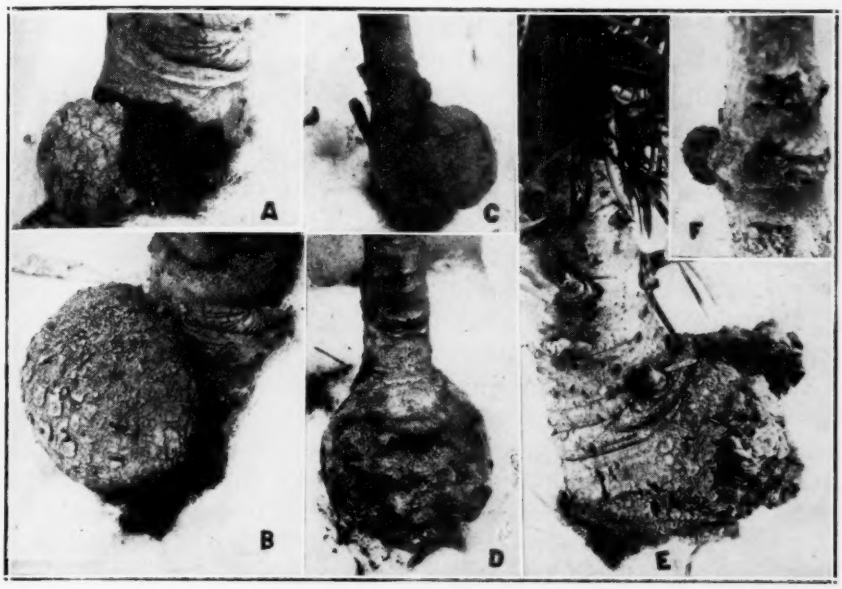

FIG. 3. Galls artificially induced on *Abies* spp. (Pinaceae) by inoculation with the crown-gall organism isolated from *Salix* sp. A and B. Gall on *Abies holophylla* (SPI 90649): A, 18 months after inoculation; B, after 52 months. C to E. Galls on *A. concolor:* C, 30 months after inoculation; D, after 65 months; E, after 60 months. F. Gall on *A. firma*, after 11 months.

SUMMARY

Inoculations with *Phytomonas tumefaciens* (Smith and Townsend) Bergey *et al.* were made on different species of conifers. Galls developed on the following species: (Taxaceae) *Podocarpus elongata, Taxus baccata* var. *erecta, T. brevifolia, T. media, Torreya californica;* (Taxodiaceae) *Cunninghamia lanceolata, Sequoia gigantea, S. sempervirens, Sciadopitys verticillata;* (Pinaceae) *Abies cephalonica, A. concolor, A. firma,* and *A. holophylla.*

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FUSARIUM YELLOWS OF BEANS

JAMES B. KENDRICK AND WILLIAM C. SNYDER

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INTRODUCTION

In 1929, Harter[1] reported a vascular fusarium disease of field beans (*Phascolus vulgaris* L.) in the Sacramento Valley, California, characterized by stunting of the plants, yellowing and dropping of the leaves, and almost complete vascular invasion by the fungus. The writers observed a similar disease in the same locality in 1929 and again in 1933. In 1934, Kendrick[2] reported the disease as fusarium yellows of beans and showed that it was seed-transmitted. A general survey of the district showed it to be confined to a few fields in a restricted area. These fields have been planted to other crops, and no additional infested areas have been observed in this district.

The disease was not observed again until the summer of 1940, when it was found in 2 large plantings of pink beans in the upper Sacramento Valley, California. One field of approximately 50 acres showed an estimated damage of 50 per cent, while the other showed only single isolated diseased plants. The severity of the disease and information secured from the grower indicated that it had been present in the severely diseased field for several years. In 1941, isolated diseased plants were observed in another field in this same district.

The presence of isolated infected plants in the one field in 1940 and in another in 1941 indicated introduction of the disease by seed-borne fungus spores. A rather careful examination of these two fields failed to show other than single diseased plants, usually in widely separated areas.

Repeated isolations from discolored vascular tissue of diseased plants consistently yielded the same fungus, which, in culture, resembled the cowpea (*Vigna sinensis* Endl.) wilt organism, *F. oxysporum* f. *tracheiphilum* (E. F. S.) Sny. and Hans.

SYMPTOMS

The disease first shows as a slight fading from green to yellow of the lower leaves on one side of the plant. This change from green to yellow progresses rapidly from the lower leaves upward and is in most cases much more pronounced on one side of the plant. The bright yellow leaves later fall from the plant, while the normal green to pale-green ones on the opposite side remain attached. In the later stages of the disease many plants are entirely killed, while others may retain a few sickly leaves on one side of the plant and a weak terminal growth until maturity.

The vascular system shows a dark-brown discoloration extending into the

[1] Harter, L. L. A Fusarium disease of beans. (Abstract) Phytopath. **19**: 84. 1929.

[2] Kendrick, James B. Seed transmission of Fusarium yellows of beans. (Abstract) Phytopath. **24**: 1139. 1934.

main stem, lateral branches, petioles, and peduncles. The vascular browning is typical of that occurring in the fusarium wilt of cowpeas.

PATHOGENICITY

Because of the similarity of symptoms of bean yellows and those of cowpea fusarium wilt (*Fusarium oxysporum* f. *tracheiphilum*) reciprocal inoculations were conducted along with the pathogenicity tests. For these tests, pure cultures of the bean yellows Fusarium and that obtained from cowpea-wilt-infected plants were grown on steam-sterilized oats, thoroughly mixed with steam-sterilized soil. In all trials, noninoculated steam-sterilized soil was used for controls. The soil was placed in 6-inch pots and the seed planted immediately. All pots were held in a greenhouse where the temperature was maintained at 70° F. or above. In these studies 2 species of beans, pink and red Mexican (*Phaseolus vulgaris*), and Henderson's bush Lima and Wilbur Lima (*Phaseolus limensis* Macf. var. *limenanus*, Bailey), and blackeye cowpea (*Vigna sinensis*) were used.

On November 3, 1929, 8 isolates of cowpea fusarium wilt and 6 isolates of Fusarium from bean yellows were separately inoculated into sterilized soil. Of the 48 pots of cowpea-wilt-inoculated soil, 16 were planted with blackeye cowpeas, 16 with pink beans, and 16 with Henderson's bush Lima beans. Of the 60 pots inoculated with Fusarium from bean yellows, 12 were planted with blackeye cowpeas, 12 with pink beans, 12 with red Mexican beans, and 12 with Henderson's bush Lima beans. On January 21, 1930, final records were taken on diseased and healthy plants. Plants showing no obvious leaf symptoms were pulled and examined for vascular invasion. Those plants showing vascular invasion and no external plant symptoms were cultured to determine the cause of the vascular discoloration (Table 1).

TABLE 1.—*Summary of cowpea fusarium wilt and bean yellows Fusarium cross-inoculation trials of Nov. 8, 1929, to Jan. 21, 1930*

Source of culture used	Blackeye-cowpea		Pink beans		Red Mexican beans		Henderson's bush Lima beans	
	Number of plants	Percentage diseased	Number of plants	Percentage diseased	Number of plants	Percentage diseased	Number of plants	Percentage diseased
Cowpea wilt	193	24.9	225	0.0	.....	.....	184	0
Bean yellows	211	0.0	231	14.2	238	25.8	206	0
Control	121	0.0	121	0.0	131	0.0	146	0

The above table shows that the cowpea-fusarium-wilt fungus did not attack either of the bean varieties, and the Fusarium causing bean yellows did not attack either blackeye cowpeas or Henderson's bush Lima beans.

On February 12, 1934, a similar series of greenhouse experimental plantings were made in which 3 isolates of cowpea fusarium wilt and 3 of Fusarium from bean yellows were used. In this series, blackeye cowpea,

pink beans, and Wilbur Lima beans were used. On March 19, 1934, final records were taken and the summarized results are presented in table 2.

TABLE 2.—*Summary of cowpea fusarium wilt and bean yellows fusarium inoculation trials of February 12 to March 19, 1934*

Source of fungus used	Blackeye cowpea		Pink beans		Wilbur Lima beans	
	Number of plants	Percentage diseased	Number of plants	Percentage diseased	Number of plants	Percentage diseased
Cowpea	78	82.0	150	0.0	94	0
Cowpea	58	96.5	95	0.0	67	0
Cowpea	54	74.1	91	0.0	77	0
Pink bean	107	0.0	150	78.0	133	0
Pink bean	98	0.0	79	64.5	56	0
Pink bean	90	0.0	123	88.5	96	0
Controls	115	0.0	107	0.0	98	0

Table 2 shows a much higher percentage of infection than does table 1. This probably was due to the higher average greenhouse temperature and better light conditions prevalent during these tests. It further shows that the bean fusarium does not attack cowpea, the cowpea fusarium does not attack beans, and that Lima beans resist the attack of both organisms.

In the summer of 1929, 17 varieties of beans, 3 of Lima beans, and 3 of soybeans (*Soja max* Piper) were planted in a field plot naturally infested with the cowpea fusarium in Stanislaus County, California. The plants were observed throughout the summer for evidence of disease and no disease occurred. Susceptible cowpea selections planted in the same plot showed a high percentage of wilted plants.

Again in 1930, 10 varieties of beans, 3 of Lima beans, and 3 of soybeans were included in a cowpea-wilt experimental test on heavily infested soil in the same county. A careful check throughout the season failed to reveal any evidence of wilt on the bean, Lima bean, or soybean varieties, whereas the susceptible cowpea varieties were 100 per cent killed by wilt.

In 1940, a field of approximately 50 acres of pink beans showed more than 50 per cent severely diseased plants from fusarium yellows. This same field was planted to Wilbur Lima beans in 1941, and there was no evidence of the disease in any of the plants.

Thus, extensive greenhouse inoculation tests, field experiments, and field observations show that *Fusarium oxysporum* f. *tracheiphilum*, which causes cowpea (*V. sinensis*) wilt, does not attack soybean (*S. max*), contrary to statements found in literature,[3] nor are common beans (*P. vulgaris*) or Lima beans (*P. limensis* var. *limenanus*) affected by the cowpea Fusarium. Furthermore, these data show that the bean yellows Fusarium, which attacks varieties of *P. vulgaris*, does not affect the varieties of cowpea, soybean, or Lima bean reported upon here. These facts are in line with the recognized tendency of the *formae* of *F. oxysporum* to be rather narrowly specialized,

[3] Cromwell, R. O. Fusarium blight, or wilt disease, of the soybean. Jour. Agr. Res. [U.S.] 8: 421–440. 1917.

usually to single genera. They show that not only are the vascular fusarium wilts of *V. sinensis* and *P. vulgaris* caused by separate and distinct physiologic forms of *F. oxysporum*, but, also, they suggest that the vascular fusarium disease of *S. max* may be caused by still another and distinct form.

TAXONOMY

Single-spore cultures of the fungus recovered from tissue platings of the discolored vascular elements of diseased bean (*Phaseolus vulgaris*) plants proved to be morphologically in agreement with *Fusarium oxysporum* Schl., as emended by Snyder and Hansen.[4]

Since a form of *Fusarium oxysporum*, pathogenic on bean (*P. vulgaris*), has not been described heretofore, and, since data given herein indicate that the form from bean is a specialized pathogen of this host, it is proposed that this pathogen be distinguished from other forms of *F. oxysporum* by the creation of a new form name as follows:

Fusarium oxysporum f. **phaseoli,** n. f. A vascular parasite of *Phaseolus vulgaris* causing Fusarium yellows of beans.

CONTROL

Kendrick,[2] in 1934, showed that the *Fusarium* causing bean yellows is transmitted with the seed, probably by spores of the fungus adhering to the seed coat. He further showed that dusting the seed with 8 oz. of Semesan or 4 oz. of Ceresan per 100 lb. of seed completely eliminated the disease in greenhouse tests. Since the causal fungus will persist in the soil for long periods of time, it is recommended that seed from a suspected diseased area be dusted with Semesan or Ceresan before being planted in a disease-free area. Fields infected with the bean yellows fungus can be safely planted to Lima beans, soybeans, or cowpeas.

SUMMARY

A vascular fusarium disease of field beans was first observed in the Sacramento Valley, California, in 1929 and again in 1933. Due to the absence of bean plantings in the infested area, the disease was not observed again until 1940, when it was observed in another section of the valley.

The disease is characterized by a gradual yellowing of the leaves from the lower leaves upward, dwarfing of the plants, and eventual dropping of the leaves, and death of the plant in many cases. The vascular system of the stem and leaf petioles shows a dark brown discoloration.

A *Fusarium*, which in culture resembled the cowpea wilt organism was consistently isolated from diseased bean tissue.

Inoculation studies showed the bean *Fusarium* to be pathogenic to varie-

[4] Snyder, W. C., and H. N. Hansen. The species concept in *Fusarium*. Amer. Jour. Bot. 27: 64–67. 1940.

ties of common beans (*Phaseolus vulgaris*) but not to Lima beans (*P. limensis* var. *limenanus*), cowpea (*Vigna sinensis*), or soybeans (*Soja max*).

Since field, greenhouse, and cultural studies have shown that bean yellows is caused by an apparently undescribed form of *Fusarium*, it is herein designated as *F. oxysporum* f. **phaseoli** n. f.

The causal *Fusarium* may be transmitted with the seed, and infection can be controlled by seed treatment with Semesan or Ceresan.

Lima beans, cowpeas, and soybeans can be safely planted in bean-yellows infested soil.

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EFFECTIVE METHODS OF INOCULATING SEED BARLEY WITH COVERED SMUT (USTILAGO HORDEI)[1]

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(Accepted for publication March 5, 1942)

INTRODUCTION

Covered smut of barley, *Ustilago hordei* Lagerh., causes considerable loss, much of which can be avoided by growing smut-resistant varieties. Progress in this direction, however, has long been hampered by the difficulty in getting high percentages of smutted heads in susceptible varieties through artificial inoculation of the seed. Since Jensen's (8) report in 1888, it has been repeatedly observed that when seed from a smutted crop is threshed, stored, and then sown without treatment, high percentages of covered smut frequently occur. Paradoxically, however, when clean seed is artificially inoculated by superficially blackening it with millions of spores, only low percentages of smutted heads usually occur (2, 3, 15, 16). This has been difficult to understand in view of the belief that spores of covered smut remain enclosed in the smutted heads until threshing, when the smutted heads are disintegrated and spores come in contact with the surface of seed. There would seem to be little important difference between this method of inoculation and the artificial one of shaking or rolling seeds in smut spores.

In an attempt to clarify this problem, the senior writer (15) studied inoculation as it occurs in the usual culture of barley. It was found that spores are released from smutted heads in the field from heading to threshing, as well as during threshing. Many spores and extensive ramifications of mycelium from germinated spores were found beneath the hulls of naturally inoculated seed obtained from different parts of the United States. This subhull inoculum, moreover, proved especially effective in infection. It also has been observed repeatedly in earlier investigations that the blackening of seed with smut spores is far more effective in smut production if the hulls are first removed (2, 4, 9, 16). It is evident, therefore, that the hulls interfere with infection when they come between the inoculum and caryopsis of the kernel. Manual dehulling of the seed is too slow and tedious for practical use, and no satisfactory mechanical or chemical means of dehulling has been available (9). On the basis of these facts the writers devised and studied several new methods of seed inoculation. Some of the new methods have given excellent results, year after year, under a wide variety of climatic conditions. As a result, the study of physiologic races

[1] Cooperative investigation of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, the New York (Cornell) Agricultural Experiment Station, and the Idaho Agricultural Experiment Station. Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 191.

[2] Pathologist and Associate Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

in *Ustilago hordei* (13) and breeding for resistance against the races that have been isolated are now going forward.

EARLIER INVESTIGATIONS

Tapke (15) has recently reviewed the earlier studies on natural and artificial inoculation of seed barley with covered smut.

METHODS AND MATERIALS

It has been shown in recent years that the baneful effects of a poor method of seed inoculation may be largely or entirely offset by growing the plants under greenhouse conditions after emergence (14). For this reason the tests in the present study were conducted under field conditions.

The new seed-inoculation methods described herein were patterned after Haarring's (5) successful "evacuation" method for inoculating oats with loose smut (*Ustilago avenae*). In this method 1 g. of spores and 250 g. of oats are added to 1000 cc. of a special nutrient solution. The whole is stirred and evacuated for 20 minutes by suction from a water jet and the inoculated seed is spread to dry for 24 hours. The seed is then placed on moist filter paper in a chamber at 22–25° C. for 20 hours. Finally, it is dried and used at once, or stored for later use. By this method the smut spores are placed beneath the hulls close to the point of attack, as in the natural inoculation of seed. Haarring's method, while readily adaptable to a few large batches of grain, appeared too complicated for studies of physiologic races of *Ustilago hordei* involving the inoculation of several thousand samples of seed. Many simplified modifications, therefore, were tested in extensive preliminary trials and two promising methods finally were chosen for further study. These are referred to herein as the "spore-suspension" and "vacuum" methods. In the former, described previously (11), machine-threshed seed is placed in vials and covered for 15 minutes with a spore suspension made by adding 1 g. of spore dust to 1000 cc. of water.[3] During the first half-minute of this period the vials are vigorously shaken to wash spores beneath the hulls. The suspension is then decanted and the vials are inverted on clean pieces of blotting paper to absorb all free water. Next, the vials of moistened inoculated seed are packed in a tightly covered tin box, floored with a moistened blotter to maintain high humidity and promote spore germination. After 16 to 20 hours at 20° C., the vials are removed from the box, the seed is transferred to small envelopes, crimped to remain wide-open, and left for 3 or 4 days or until the seed is thoroughly dry. It is then ready to sow or to store for later use. The spore-suspension method, as thus applied, therefore, eliminates the nutrient solution,[4] the

[3] Preparation of the suspension with spores from hard, dried heads of covered smut may be facilitated by first cutting the smutted heads into small pieces with scissors. Water, sufficient to cover the pieces is then added, and the smut is soaked until it is soft. The smut masses are then mashed and water is added to make up the desired amount of suspension. The suspension is then poured into a clean container through a double layer of cheesecloth to strain out remnants of the smutted heads.

[4] Western (17) used aqueous and nutrient suspensions of oat-smut spores to inoculate

vacuum treatment, and the 24-hour preliminary drying period of Haarring's method (5).

Procedure in the vacuum method is the same as that in the spore-suspension method, except that after the seed is put in vials and covered with the spore suspension, the vials are placed in a special jar and subjected to 30 inches of vacuum for 15 minutes. The spore suspension is then decanted and the seed is incubated for 16 to 20 hours and finally put into envelopes and left to dry as in the spore-suspension method.

In the study of the spore-suspension and vacuum methods, the surface dusting method of seed inoculation also was used for comparison. This was applied by shaking seed and spores in a vial until the seed was thoroughly blackened. The inoculated seed then was removed from the vial and momentarily shaken on a fine screen to remove the excess of smut spores.

The field plantings were made in 1936 and 1937 at Moscow, and Sandpoint, Idaho, and Ithaca, New York, in 5-foot rows spaced 1 foot apart. Each year a single lot of smut was used for all of the inoculations. It consisted of a physiologic race of *Ustilago hordei* to which the varieties used in the tests were susceptible. These varieties were Hannchen (C.I. 531), Odessa (C.I. 934), and Trebi (C.I. 936).

RESULTS

In the first test, conducted in 1936, all of the seed, previous to inoculation, was treated with formaldehyde solution (1 to 320) for 1 hour. The treated seed was then immediately rinsed in running water for one-half hour and spread out in a thin layer to dry until restored to its original pre-treatment

FIG. 1. Trebi barley showing (left) an untreated kernel with hulls intact and (right) four kernels with hulls loosened and split as a result of treatment with formaldehyde solution for 1 hour followed by washing in water and drying.

weight. In studies of physiologic races of *Ustilago hordei* such treatment of the differential seed lots is necessary or desirable to insure against possible foreign inoculum. As a result of treatment and drying, the hulls frequently split (Fig. 1) and become more or less loose around the caryopsis, due, probably to dissolution of the sticky substance noted by Harlan (6) that

oats, and noted that the suspending medium had little effect on spore germination or promycelial growth on the oats.

causes the glumes to adhere to the kernels. This facilitates the lodgment of spores beneath the seedhulls and as noted later (Table 2), markedly increases the effectiveness of inoculation. The seed was sown in duplicate sets at Ithaca, N. Y., and in quintuplicate sets at Moscow and at Sandpoint, Ida. (Table 1). As shown in the table, the average percentages of smutted heads

TABLE 1.—*Comparative effectiveness of three methods of inoculating seed barley with covered smut (Ustilago hordei) at three experiment stations in 1936*

Variety	Location	Method of inoculation					
		Seed dusted with dry spores		Spore-suspension		Spore-suspension under vacuum	
		Total heads	Smutted heads	Total heads	Smutted heads	Total heads	Smutted heads
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Hannchen	Moscow, Ida.	1,890	14.9	1,626	39.3	1,826	36.2
"	Sandpoint, Ida.	448	23.4	633	55.9	454	50.7
"	Ithaca, N. Y.	200	9.0	177	33.3	165	41.8
Odessa	Moscow, Ida	1,346	18.9	1,375	55.8	1,440	66.2
"	Sandpoint, Ida.	422	36.3	519	70.5	333	74.8
"	Ithaca, N. Y.	160	10.6	227	39.2	231	42.9
Trebi	Moscow, Ida.	971	12.0	956	38.0	869	42.0
"	Sandpoint, Ida.	287	15.0	382	50.5	252	67.1
"	Ithaca, N. Y.	237	6.3	268	23.5	194	30.9
Total or average		5,961	16.8	6,163	46.9	5,764	49.5

for the 3 varieties at the 3 places were as follows: Superficial dusting method 16.8 per cent, spore-suspension method 46.9 per cent, and vacuum method 49.5 per cent.

In 1937, the experiment was conducted along similar but more comprehensive lines. In order to determine the rôle of the loosened seedhulls resulting from seed treatment with formaldehyde solution followed by washing in water and drying, all 3 inoculation methods were applied to non-treated as well as formaldehyde-treated seed. Also, a part of each inoculated seed lot was treated with 50 per cent copper carbonate dust immediately before the seed was sown.

The experiment was sown in triplicate at Moscow, Ida., and Ithaca, N. Y., and in duplicate at Sandpoint, Ida. The results were similar at all three stations and are combined in Table 2.

The data show that the pre-treatment of seed with formaldehyde solution, followed by thorough washing and drying before seed inoculation, resulted in almost a doubling of the average percentages of smutted heads from the 3 different methods of inoculation. It also resulted in relatively more effective control with copper carbonate dust. Apparently the loosening of hulls around the caryopsis enabled more inoculum and more copper carbonate to get beneath the hulls, thus increasing the effectiveness of inoculum in infection and of copper carbonate in control. The copper carbonate treatment also was far more effective when applied to seed inoculated by the

TABLE 2.—*Average covered smut infection in three varieties of barley inoculated and treated by different methods and sown at three experiment stations in 1937*[a]

Method of seed inoculation	Seed treatment		Hannchen		Odessa		Trebi		All varieties	
			Heads		Heads		Heads		Heads	
	Before inoculation	After inoculation	Total	Smutted	Total	Smutted	Total	Smutted	Total	Smutted
			Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Noninoculated check	None	None	.....[b]	Tr.[c]	.....	0.0	.....	Tr.	.....	Tr.
Seed blackened with dry spores	"	"	2392	9.7	1652	15.6	1422	6.5	5466	10.6
" " " " "	"	Copper carbonate	2415	1.2	1579	2.5	1442	1.5	5436	1.7
Spore suspension	"	None	2268	20.9	1403	29.1	1378	13.8	5049	21.2
" "	"	Copper carbonate	2357	18.2	1629	20.1	1353	16.9	5339	18.4
Spore suspension under vacuum	"	None	2451	23.4	1701	26.5	1346	21.0	5498	23.8
" " " "	"	Copper carbonate	2306	20.8	1669	24.6	1466	17.1	5441	20.9
Noninoculated check	Formaldehyde solution	None	.....	0.0	.....	0.0	.....	0.0	.....	0.0
Seed blackened with dry spores	" "	"	2335	14.9	1415	26.7	1332	12.6	5082	17.6
" " " " "	" "	Copper carbonate	2473	0.4	1493	0.7	1471	0.2	5437	0.4
Spore suspension	" "	None	2337	34.0	1760	47.5	1428	35.7	5525	38.8
" "	" "	Copper carbonate	2468	22.4	1591	20.4	1452	17.2	5511	20.5
Spore suspension under vacuum	" "	None	2393	35.6	1689	45.8	1422	38.5	5504	39.5
" " " "	" "	Copper carbonate	2298	20.3	1315	20.2	1405	19.1	5018	19.9

[a] Data are totals and averages of results at Moscow and Sandpoint, Ida., and Ithaca, N. Y.
[b] Total heads in checks, not counted.
[c] Tr. = trace, approximately 0.1 per cent smut.

surface dust method than when applied to seed inoculated by the spore-suspension and vacuum methods. In 1937, as in the previous year, the spore-suspension and vacuum methods were approximately equally effective, and both were far superior to the surface dust method.

During the past 7 years the senior writer has used the spore-suspension method in studies of physiologic races of *Ustilago hordei* (13) and in further inquiry into the practicability of the method and its effectiveness under different field conditions. The experiments, conducted at Ithaca, N. Y., involved the inoculation of 2,000 or more individual lots of seed each year. Although the inoculated barley was subjected to extremes of drought, precipitation, or heat during this period, the infections each year have been adequate for the differentiation of physiologic races. In the susceptible variety, Odessa (C.I. 934), the maximum of smutted heads in the 7 years ranged from 48 to 69 per cent with an average for all years of approximately 59 per cent. The method also proved practicable. The fact that it is a wet method is a distinct advantage in that spores of the different smut collections under test are kept from flying about and becoming a source of contamination. The writers have not used the vacuum method in extensive tests for physiologic races; but, from the experience of the present study, it would appear more difficult to apply. Placing in the vacuum jar the vials containing seed covered with spore suspension requires time. Also, it involves the hazard of contaminations through spattering of the spore suspensions when the vacuum is applied. In large-scale inoculations involving many individual smut collections, the spore-suspension method, therefore, would seem more practicable.

A few additional reports on the methods used herein, or somewhat similar methods, are available. At Sandpoint, Ida., according to Hungerford (7), the vacuum and spore-suspension methods of seed inoculation, respectively, resulted in 82 and 70 per cent of covered smut. Allison (1) and Leukel (10) also used vacuum methods that produced better results than did surface dusting of seed with dry spores. Tapke (12) obtained satisfactory results with the spore-suspension method in inoculating barley with the seedling-infecting loose smut *Ustilago nigra*. In winter barleys, up to 83 per cent of smutted heads were obtained.

It is evident from the foregoing results that relatively simple and consistently effective methods of inoculating seed barley with covered smut are finally available. Studies on physiologic races of *Ustilago hordei* and breeding for resistance in barley are now going forward.

SUMMARY

Two methods of inoculating seed barley with covered smut, the spore-suspension and vacuum methods, were devised and tested under field conditions at Moscow and Sandpoint, Ida., and Ithaca, N. Y. Both proved far more effective in smut production than the well-known method of inoculating seed by coating the surface with spores.

The spore-suspension and vacuum methods involve three essential features: (1) The seed is first treated for an hour with formaldehyde solution, then washed in water, and dried. This treatment eliminates superficially borne foreign inoculum, loosens the hulls around the caryopsis, and materially increases the effectiveness of inoculation; (2) the seed is covered with spores in suspension. Spores are thus carried beneath the hulls and come to lie close to the point of attack as in the effective natural inoculation; (3) inoculated seed is stored 16 to 20 hours while moist. This promotes spore germination and spread of inoculum before the seed is dried and sown.

The vacuum method has been slightly superior to the spore-suspension method in smut production but with large scale inoculations, particularly in studies of physiologic races, the latter method appears to be easier and safer to apply.

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CONTROL OF THE COMMON MOSAIC DISEASE OF TOBACCO BY BREEDING[1]

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Fifteen years ago it was found that the common mosaic of tobacco could be controlled successfully by insisting that the hands of the workers who chewed or smoked barn-cured tobacco be freed from mosaic virus before weeding and pulling plants,[2] but, because of difficulties where hired help is used, growers are not always successful. It is, therefore, highly desirable that resistant varieties of tobacco, equal in other respects to those commonly grown, be produced. With this object in mind, the writer has conducted breeding studies on Burley and dark tobacco in an attempt to produce desirable mosaic-resistant varieties. Three promising lines are being followed: (1) hybridizing desirable varieties with *Nicotiana digluta* (seed obtained from R. E. Clausen, University of California), and repeatedly backcrossing; (2) hybridizing desirable varieties with Ambalema and repeatedly backcrossing; (3) repeatedly backcrossing the best Ambalema-type resistant plants (A) on glutinosa-type resistant plants (N) to combine the two types of resistance.

Recently, objections have been raised to the use of the N factor in control of tobacco mosaic,[3] but in the experience of the writer the conclusion that "it does not seem that the glutinosa type of mosaic resistance has any practical value" seems entirely unwarranted.

Theoretically, the glutinosa (N) type of resistance, when transferred to commercial varieties of tobacco, should be ideal. It is governed by a dominant gene; consequently, backcrosses can be made on selected plants in every generation. Thus is should be a relatively simple matter, if the N gene has been transferred to a tabacum chromosome, as Holmes seems to believe,[4] to introduce resistance of this type into any number of commercial varieties of tobacco. The backcrossed strains, after about the third or fourth backcross, usually appear nearly identical with the commercial variety used as the backcross parent. It should then be necessary only to self a strain that has been repeatedly backcrossed and select plants homozygous for the N factor. The original variety plus the resistance factors should result. The writer has isolated 3 homozygous strains from a fifth backcross strain prepared by Holmes in which Ky. 16 was used. Two of the NN strains were of lighter color than Ky. 16, had smaller upper leaves,

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Valleau, W. D., and E. M. Johnson. Observations and experiments on the control of true tobacco mosaic. Kentucky Agr. Expt. Stat. Bull. 280, 143–174. 1927.

[3] Clayton, E. E., and H. H. McKinney. Resistance to the common mosaic disease of tobacco. Phytopath. 31: 1140–1142. 1941.

[4] Holmes, F. O. Inheritance of resistance to tobacco-mosaic disease in tobacco. Phytopath. 28: 553–560. 1938.

and bloomed much lower. The suckers were very tough and hard to remove. The third strain was of the same color as Ky. 16, grew nearly as large, but yielded about 25 per cent less tobacco. Several other homozygous strains of Burley of the writer's own breeding have been isolated but nearly all have been discarded because of slow growth, low yield, or other undesirable characters. These results seem to indicate that when the N factor from *Nicotiana glutinosa* is well established in the tabacum genom, it still carries with it other factors that, when in a homozygous condition, markedly affect the type of plant produced. Some NN strains appear desirable, suggesting that, eventually, satisfactory varieties may evolve.

As to the value of the N factor in the control of tobacco mosaic, there seems little doubt, in spite of objections that have been raised to it. There is no question that seedlings transplanted to thumb pots usually will be destroyed if heavily inoculated, making it necessary to remove the inoculated leaf soon after the necrotic spots develop, if the plant is to be saved. Rapidly growing plants in the field may likewise be destroyed if *heavily* inoculated. In the writer's field tests the past season, 27 strains of Nn or NN Burley tobacco were inoculated June 18, 1941, 25 days after setting. Inoculum used consisted of freshly crushed green leaves, applied with the thumb and fingers to the tip of one upper leaf. Of 441 N plants, 435 developed necrotic spots but otherwise remained healthy (Nn or NN); only 6 developed systemic necrosis Nn or NN). Inoculation was undoubtedly heavier than would ordinarily occur in accidental inoculation by the farmer. In addition, 3 NN strains of Burley were tested extensively with farmers in 1940 and 1 strain in 1941. These strains were not introduced for general planting, but were used for the purpose of demonstrating that mosaic can be controlled by the use of a resistant variety. In some of these demonstrations, the grower inoculated both the resistant and susceptible plants, but in no instance has a case of systemic necrosis been reported. An occasional grower, who has been much troubled by mosaic in the past, has grown a crop of *NN* tobacco with complete success as far as mosaic is concerned.

Another point should be kept in mind in connection with the use of the necrotic response in mosaic control: Nearly all mosaic infection originates on the hands of the workers, either as a result of handling viruliferous dried tobacco or, in rare cases, handling diseased weeds in the plant bed.[5] Assuming, for argument's sake, that every plant, inoculated with tobacco mosaic virus at weeding, pulling, and setting time, develops systemic necrosis and dies, experience with susceptible varieties has shown that it would be rare indeed that 10 per cent of the plants in the field would be affected following setting. Field evidence indicates that there would be no further spread from necrotic plants the remainder of the season because of low virus content in necrotic plants that may survive. The crop at harvest time would be virtually virus-free, and would not, therefore, carry virus over winter.

[5] Valleau, W. D., and E. M. Johnson. Tobacco mosaic—sources of infection and control. Kentucky Agr. Exp. Stat. Bull. 376. 1937.

Therefore, any chewing or smoking tobacco from the crop, or any trash used for fertilizer from it, would be virus-free, and the second-year crop should be entirely uninjured by the virus. Granting that soil carry-over may sometimes be a minor factor in field infection, there should be none following an N-resistant crop because of the few plants affected and because of the very low virus content of the necrotic plants. In warm regions, where tobacco plants sometimes survive the winter and act as a source of mosaic for the succeeding crop, there is little likelihood that necrotic plants would survive. The mosaic disease in an N-resistant crop thus is self-eradicatory in contrast with its self-perpetuating habit in a susceptible crop.

With Burley tobacco it has been possible to backcross with a susceptible variety 4 consecutive times on Ambalema-resistant F_2 Burley plants and still maintain resistance. There is some question as to whether resistance is of as high a degree as occurred in the original Ambalema selections. Backcross resistant seedlings develop some mottling 2 or 3 weeks after inoculation and may become slowly and nearly completely invaded if grown to maturity; yet the growing-point leaves of rapidly growing plants are unmottled and undistorted, indicating a high degree of resistance. When plants of this degree of resistance are grown in the field and inoculated with a bleaching strain of the mosaic virus as soon as rapid growth commences, the majority remain healthy, except for local chlorotic lesions, while a part develop an occasional chlorotic ring pattern on one or more lower uninoculated leaves. In commercial plantings where mosaic is abundant in a susceptible variety, these resistant varieties remain entirely free from any noticeable infection. The objections to these resistant Burley varieties, noted so far, are that they have a somewhat objectionable plant type, even after repeated backcrossing; yield has been lower than is obtained from the Burley parent (Ky. 16) used in backcrossing; and the majority of the hybrids wilt in hot weather, with the result that one or more leaves scald. Ky. 16 seems to be completely free from this trouble. It is possible that these objections may be overcome. The quality of certain of the resistant strains appears to be satisfactory.

Theoretically, there may be some objections to the A type of resistance in that resistant plants sometimes carry a slight amount of virus, which might act as a source of inoculum for tomatoes or other susceptible crops when the viruliferous tobacco is prepared and sold in commercial form. Actually such a large percentage of A-resistant plants escape systemic infection, when heavily inoculated in the field, that there is little danger of any resistant plants developing systemic infection under farm conditions. There is a real danger, however, that strains of the mosaic virus may develop that will become systemic in N plants and produce a mottle disease, rather than necrosis, as Blood and Watson report in *Datura meteloides*.[6] Both of these

[6] Blood, H. L., and R. D. Watson. A modification of the tobacco mosaic virus No. 1 occurring in *Datura meteloides*. Utah Acad. of Sci., Arts and Letters **15**: 15–19. 1938.

theoretical difficulties can be overcome, and nearly immune strains of tobacco produced, if the N and the A types of resistance are combined in one variety. Ten such strains of Burley were set in the field in 1941 and inoculated 25 days after setting. All were heterozygous for N, and had not been selected for A-type resistance, except that A-type resistant plants were used in backcrossing. Of a total of 294 plants inoculated, 245 showed no evident signs of infection, 46 developed systemic mottle mosaic, and 3 developed a few chlorotic ring patterns on lower leaves. In tests later in the summer it was usually difficult to detect N plants because of slow development of local necrotic spots in plants in which the 4 recessive *a* factors also were present. In the greenhouse, tender *Nn aaaa* plants develop local necrotic spots but rarely manifest systemic necrosis under conditions where nearly all Nn plants would be destroyed. In a few instances *Nn aaaa* plants that developed systemic necrosis recovered completely when set in the ground bench.

From the writer's experience it seems safe to conclude that both the Ambalema (A) and the glutinosa (N) types of resistance, either singly or together, will prove satisfactory for practical control of tobacco mosaic if satisfactory commercial varieties containing these factors in a homozygous condition can be produced.

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THE COMPOSITION AND FIELD PERFORMANCE OF SOME SILVER SPRAYS

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During the first fifteen years of the present century several attempts were made to use various silver compounds and mixtures as plant sprays. Though there was no consistent success with any of these, probably the most promising was a silver nitrate-soap mixture.[3] An effort has been made recently by the senior writer[4] to develop fungicidal sprays with silver as the toxic component. These developmental studies were conducted under laboratory and greenhouse conditions. It is the purpose of this paper to present the composition and field performance of some of the more promising silver sprays developed in the laboratory.

THE SILVER SPRAYS

One of the promising mixtures was prepared by adding a silver nitrate solution to a solution of sodium lauryl sulphate.[5] This spray will be referred to as the silver-lauryl sulphate mixture. The silver nitrate concentration in this mixture varied between 0.299 and 1.195 g. per gal., while the sodium lauryl sulphate concentration (as Dreft or IN–181–P) varied from 6.3 to 7.5 g. per gal. Two other silver sprays had 3 components. They contained, in common, silver nitrate and hydrated lime. The third component in each case was either ferrous sulphate or manganous sulphate. Laboratory studies indicate that the following relative concentrations of the 3 components produce mixtures whose residues are most adherent to potato foliage: (A) the silver-ferrous sulphate mixture containing silver nitrate 0.598 g., ferrous sulphate ($FeSO_4 \cdot 7H_2O$) 0.98 g., and hydrated lime (73 per cent calcium hydroxide) 0.59 g. per gal. of spray mixture; (B) the silver-manganous sulphate mixture containing silver nitrate 0.598 g., manganous sulphate ($MnSO_4 \cdot 4H_2O$) 0.77 g., and hydrated lime (73 per cent calcium hydroxide) 0.59 g. per gal. of spray mixture.

The ferrous sulphate and manganous sulphate mixtures were each prepared by simultaneously adding the suspended hydrated lime and dissolved sulphate salt to the solution of silver nitrate. The complete addition

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[3] Vermorel, V., and E. Dantony. Le mildiou de la grappe. Prog. Agr. et Vit. **31**: 101–102. 1910; Abstract in Rev. Vit. **34**: 71. 1910.

[4] Nielsen, L. W. Studies with silver compounds and mixtures as fungicidal sprays. In press.

[5] Commercial sources of sodium lauryl sulphate:

Dreft, Orvus W. A. Paste — Procter and Gamble Co., Cincinnati, Ohio.

IN–181, IN–181–P — E. I DuPont de Nemours & Co., Wilmington, Delaware.

of the sulphate solution to the silver nitrate may precede that of the hydrated lime without any visible material change in the spray. However, the available data[6] indicate that the simultaneous addition of the sulphate solution and the hydrated lime suspension will produce the more adherent mixture. The resulting mixture in each case is black, and has an amorphous precipitate similar to that of Bordeaux mixture.

Of the 3 sprays, the silver-ferrous sulphate mixture was the most adherent to potato foliage in the laboratory tests; silver-lauryl sulphate mixture was the least adherent. Due to an excess of sodium lauryl sulphate, the silver-lauryl sulphate mixture readily wets the sprayed foliage. However, in order to wet foliage having a waxy cuticle, wetting agents must be added to the ferrous- and manganous-sulphate mixtures. Since silver ions have an affinity for proteins, wetting agents containing proteinaceous materials should be avoided.

The field experiments were concomitant with the laboratory developmental studies. Thus, in the field tests the concentrations of components of any silver spray mixture and the silver compound itself have varied somewhat from year to year as progress was made in the laboratory. For this reason the above described spray mixtures, which recent laboratory studies indicate are best, are not included in the field experiments reported here.

THE FIELD EXPERIMENTS

During the summer of 1938, the silver-lauryl sulphate mixture, silver oxide, and a colloidal-like silver, were compared with Bordeaux mixture and with red cuprous oxide (as a spray) for the control of late blight of celery (*Septoria apii*). The copper concentration in the red cuprous oxide mixture was equivalent to that in the 4–4–50 Bordeaux mixture. The silver nitrate concentration for each of the silver sprays was 0.598 g. per gal.

TABLE 1.—*Data from a field experiment comparing three silver sprays and two copper sprays for the control of Septoria apii infection of celery*

Spray	Mean number of marketable* leaves per plant	Difference† between Bordeaux mixture and other treatments
Bordeux mixture (4–4–50)	7.19	
Silver-lauryl sulphate	6.68	−0.51
Colloidal silver (a) (b)	5.23	−1.96**
Silver oxide (a) (c)	5.2	−1.99**
Red cuprous oxide	5.08	−2.11**
Control	2.55	−4.64

* Marketable leaves means those leaves either free of infection or having mild infection. Severely infected and dead leaves were counted but not considered in this analysis.

† Minimum difference for odds of:

* 19: 1 ..... 1.43 leaves

** 99: 1 ..... 1.95 leaves

(a) Lethane 1/600 was used as a spreader.

(b) Silver nitrate reduced with stannous chloride.

(c) Produced by adding silver nitrate solution to sodium hydroxide solution.

[6] See footnote 4.

The experimental plants were artificially inoculated July 11, 1938. By July 30 good infection had developed and the first spray application was made. The plants at that time were developing their 4th or 5th leaves. Each spray treatment was applied to 27 ft. of row and was replicated 4 times. Eight applications of spray were made at weekly intervals. The data were obtained by examining carefully every 4th plant in each row to determine the number of disease-free or mildly infected leaves. Both are grouped together as marketable leaves. The data are summarized in table 1. During the time of this experiment there was a total precipitation of 8.49 inches.

Of the silver sprays the silver-lauryl sulphate mixture gave best late-blight control, and the control obtained with this spray was nearly as good as that obtained with 4–4–50 Bordeaux mixture. No visible residue was left by the silver-lauryl sulphate spray and no injury was observed.

The silver-lauryl sulphate mixture at concentrations of 0.299 and 0.598 g. of silver nitrate per gal. was compared with 5–2.5–50 Bordeaux mixture for the control of late blight of potatoes during the summer of 1939. In 1940 silver-manganous sulphate with 0.299, 0.598, and 1.195 g. of silver nitrate per gal. and silver-ferrous sulphate with 0.598 g. of silver nitrate per gal. were compared with Bordeaux mixture. In both experiments the silver sprays were significantly less efficient than 5–2.5–50 Bordeaux mixture in controlling late blight.[7]

During the season of 1940 various spray materials were compared for the control of tulip "fire," caused by *Botrytis tulipae*. In previous experiments copper-containing sprays in general proved injurious to tulips. High-lime Bordeaux mixture (1.5–4.5–50) was comparatively safe, while 4–4–50 Bordeaux mixture caused serious injury to foliage with consequent reduction in bulb growth. Of the sprays tested in 1940 only the silver-lauryl sulphate, silver-manganous sulphate, and the 1.5–4.5–50 Bordeaux mixture were statistically better than the check when compared on the basis of bulb weight increase. The other sprays will not be considered further here. The silver sprays contained 1.195 g. of silver nitrate per gal. The silver-lauryl sulphate mixture contained 6.8 g. of IN–181–P (sodium lauryl sulphate) per gal. The silver-manganous sulphate mixture contained 3.79 g. of manganous sulphate and 6.8 g. of hydrated lime per gal. Each treatment was replicated 8 times. Spray applications were made April 26, May 7, May 23, and June 2, 1940, and were applied before predicted rain periods. Prevailing conditions were extremely favorable for an epiphytotic of tulip "fire."

The 2 silver sprays, Bordeaux mixture and the check, were compared on the basis of (1) bulb weight increase, (2) number of leaf spots, and (3) number of flower spots. Bulb increase was calculated by subtracting the planting weight from the harvest weight and dividing the remainder by the

[7] Unpublished data from experiments conducted by W. M. Epps, Research Assistant, Department of Plant Pathology, Cornell University, Ithaca, New York.

planting weight. The leaf-spot data were taken by using a card having an opening of 1 sq. in. The square opening was placed successively on 6 leaves chosen at random in each row of the 5 rows per plot—making a count of 30 sq. in. for each plot. The flower data were taken by counting the total number of lesions on 18 flowers selected after a definite pattern, such as flowers No. 1, 3, 6, and 9 in the first row; No. 2, 5, and 8 in the second row; and No. 1, 3, 6, and 9 in the third row, etc., through the 5 rows in each plot. The data are summarized in table 2.

TABLE 2.—*A field comparison of two silver sprays and Bordeaux mixture for the control of tulip "fire" as determined by measuring bulb weight increase, leaf spots, and flower spots of sprayed plants*

Spray	Bulb weight increase in per cent of planting weight	Leaf spots	Flower spots
	Per cent	*Number*	*Number*
(a) Bordeaux mixture 1.5–4.5–50	100.6*	17.6	143.1
(b) Silver-manganous sulphate	101.0	52.3	161.6
Silver-lauryl sulphate	99.7	55.2	143.4
Check	77.1	222.7	307.1

* Numbers given in table are means of eight replications.
(a) Spreader, Vatsol OT† at a concentration of 1/650 based on solid.
(b) Spreader, Vatsol OT at a concentration of 1/1000 based on solid.
† Commercial source:
American Cyanamid and Chemical Corp.
30 Rockefeller Plaza
New York City

The silver sprays compared favorably with the 1.5–4.5–50 Bordeaux mixture in fungicidal action and had a further advantage in that they left no unsightly residue on the foliage and flowers.

Silver-lauryl sulphate, silver-manganous sulphate and silver-ferrous sulphate sprays were compared with Bordeaux mixture during the season of 1941 for the control of tulip "fire." Owing to the dry growing season very little infection developed in the check plots and no comparative protection data were obtained.

The silver-manganous sulphate mixture, as prepared for the tulip experiments, including the spreading agent, cost 2.7 cents per gallon. The Bordeaux mixture plus spreading agent cost 1.4 cents per gallon. However, the silver spray covered about 25 per cent more area because of its superior wetting properties. With a manganous sulphate concentration of 0.77 g. per gal. as given in the earlier formula (based on laboratory studies), the cost of this spray would have been approximately 2.0 cents per gallon.

SUMMARY

The composition and method of preparing three silver sprays are given.

In a field experiment there was no significant difference between silver-lauryl sulphate mixture and Bordeaux mixture in the control of late blight

of celery. The 3 silver sprays tested were not so efficient as Bordeaux mixture for the control of late blight of potatoes. Both the silver-lauryl sulphate mixture and silver-manganous sulphate mixture were as good as Bordeaux mixture for the control of tulip "fire" in one season's test.

None of the silver sprays left an objectionable residue on the sprayed foliage. If one may judge from the experiments with tulips, the silver sprays may be considered to give greater promise of value as fungicides for certain plants that are susceptible to copper injury.

FUSARIUM WILT OF RADISH

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A vascular fusarium disease of White Chinese Winter radish (*Raphanus sativus* var. *longipinnatus* Bailey) was observed in a seed field in San Benito County, California, in April, 1934.[1] This planting had been made sometime in the summer of 1933 and the disease was well advanced, indicating that it had been present the previous summer. Several plants had been killed by the disease; many others showed a yellowing and dwarfed unilateral growth. The disease has not been observed elsewhere in California, neither has it again occurred on this ranch, as the infested area has not subsequently been planted to radishes.

The general symptoms of the disease on the large radish seed plants resembled those of cabbage yellows caused by *Fusarium oxysporum* f. *conglutinans* (Wr.) Sny. and Hans. Diseased plants showed yellowing and dropping of the leaves, frequently confined to one side of the plant, a dark-brown, vascular discoloration, severe stunting, and in many cases, death of the plant. A Fusarium was readily isolated from the discolored vascular tissue of the diseased stems.

Young plants, growing in artificially infested soil in the greenhouse, also show symptoms characteristic of cabbage yellows. In 3 to 4 weeks after seedlings emerge from the soil, a unilateral yellowing of the lower leaves begins to show on one side of the plant. The yellowing of the leaves progresses rapidly from the lower to the upper leaves, and the leaves soon drop from the plant. The invasion of one side of the petiole and leaf by the fungus often results in a lateral and downward curling of the leaf. In the later stages of the disease on young plants, fungus invasion becomes general and all the leaves usually turn yellow and drop from the plant, leaving a bare stem, which soon dies.

Pathogenicity studies were made by planting seed directly in pots of steam-sterilized soil. After the seedlings were established, agar-plate cultures of the causal Fusarium were shredded and added to flasks of sterile water. A small amount of the mycelial and spore suspension was poured around the base of the plants and immediately washed into the soil by a rather heavy watering.

On July 7, 1934, a series of such inoculations were made in which 3 separate isolates of Fusarium from radish were used and White Chinese Winter radish, White Icicle radish (*Raphanus sativus* L.) and Early Jersey Wakefield cabbage (*Brassica oleracea* var. *capitata* L.) were exposed to each isolate. The first symptoms of the disease were observed 21 days after inoculation, and final records were taken on September 18, when most of the radish

[1] Kendrick, James B., and William C. Snyder. A vascular fusarium disease of radish. (Abstract) Phytopath. **26**: 98. 1936.

plants were dead. No evidence of the disease showed on the cabbage plants. The results are summarized in table 1.

TABLE 1.—*Summarized results of greenhouse inoculation tests of radish and cabbage to the Fusarium responsible for the radish wilt*

Variety	Inoculated soil		Controls	
	Number of plants	Percentage diseased	Number of plants	Percentage diseased
White Chinese Winter radish	104	91.3	24	0.0
White Icicle radish	113	90.2	32	0.0
Early Jersey Wakefield cabbage	161	0.0	57	0.0

On September 22, 1934, 8 varieties of radish, Early Jersey Wakefield cabbage, and Jersey kale (*Brassica oleracea* var. *acephala* DC.) were planted in the same pots of soil inoculated July 7. The first symptoms of the disease were noted on the young seedlings on October 16, and final notes were recorded on December 20, 1934, when most of the plants were dead from the disease. The results are tabulated in table 2.

TABLE 2.—*Susceptibility of radish varieties, cabbage, and kale to the Fusarium wilt disease of radish in greenhouse inoculation trials*

Variety	Inoculated soil		Controls	
	Number of plants	Percentage diseased	Number of plants	Percentage diseased
White Chinese Winter radish	123	87.8	36	0.0
White Icicle radish	169	85.8	52	0.0
Long Black Spanish radish	38	71.0	7	0.0
California Mammoth White radish	62	90.3	20	0.0
Chinese Rose Winter radish	61	25.5	13	0.0
Scarlet Turnip radish	64	79.6	22	0.0
Long Scarlet radish	62	75.8	21	0.0
French Breakfast radish	62	35.3	23	0.0
Early Jersey Wakefield cabbage	217	0.0	77	0.0
Jersey kale	75	0.0	35	0.0

The above table shows that all types of radish tested are susceptible to the fusarium disease. The fact that the varieties Chinese Rose Winter and French Breakfast showed considerably less infection than the others might indicate some degree of resistance in these two varieties. Tables 1 and 2 also show that the Fusarium causing the radish disease does not attack cabbage or kale, both of which are susceptible to cabbage yellows.

Pure cultures of the radish Fusarium, prepared from single conidia, have shown the fungus to be a member of section Elegans.[2] Culturally it differs from the cabbage yellows Fusarium principally in the unreliable character of mycelial color, which, in case of the radish fungus, is usually a deep vinaceous. Morphologically the radish Fusarium falls into the species *F. oxysporum* Schl., as emended by Snyder and Hansen.[3]

[2] See footnote 1.

[3] Snyder, W. C., and H. N. Hansen. The species concept in Fusarium. Amer. Jour. Bot. 27: 64–67. 1940.

Since no vascular Fusarium pathogenic on radish has been described in the writers' knowledge and since data given here demonstrate that a form of *F. oxysporum,* biologically distinct from the cabbage-yellows organism, *F. oxysporum* f. *conglutinans,* causes a wilt of radish, it is proposed that the radish organism be described as a new form as follows:

Fusarium oxysporum f. *raphani* n. f. A vascular parasite of *Raphanus sativus,* and variety *longipinnatus,* causing fusarium wilt of radish.

SUMMARY

A vascular fusarium wilt of White Chinese Winter radish (*Raphanus sativus* var. *longipinnatus*) characterized by symptoms similar to those of cabbage yellows caused by *Fusarium oxysporum* f. *conglutinans* occurred in a radish seed field in California in 1934. Pathogenicity studies have shown the causal fungus to be pathogenic to common radishes (*R. sativus*), as well as White Chinese Winter radish, and distinct from the cabbage yellows Fusarium. The designation *Fusarium oxysporum* f. **raphani** n. f. is proposed for the radish wilt organism.

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PHYTOPATHOLOGICAL NOTE

Chloropicrin as a Disinfectant for Plant Beds.—The rapid accumulation of parasitic fungi in the soil in greenhouse or outdoor plant beds, as a result of repeated plantings of the same crop, often halts further work with that crop until such soil is either disinfected or replaced. The use of steam for disinfection is cumbersome and frequently not feasible. Disinfection with some of the chemicals, recommended for this purpose, involves objectionable drenching of the soil; others are inflammable, or explosixe, and most of them render the soil unfit for planting for a considerable period. Soil replacement is laborious and often unsatisfactory.

Chloropicrin, apparently free from most of these objectionable features, has been found effective and satisfactory as a soil disinfectant by a number of investigators. Clayton[1] found it effective in tobacco beds against the black root-rot fungus, *Thielaviopsis basicola*, and the root-knot nematode, *Heterodera marioni*. Cooke[2] applied it to the soil at the rate of 200 lb. per acre and prevented injury to sugar cane by *Pythium aphanadermatum*. At the rate of 400 lb. per acre, Godfrey[3] found it effective as a fungicide for species of *Fusarium, Rhizoctonia, Sclerotium*, and certain other fungi. McLaughlin and Melhus[4] improved stands and increased yields of 6 field crops, presumably by controlling harmful soil fungi with an application of chloropicrin at the rate of 480 lb. per acre. Young[5] reported that from 300 to 600 lb. per acre prevented injury from *Fusarium lycopersici* and *Heterodera marioni* in tomatoes and also controlled weeds.

In the winter of 1940–41, the writer tried chloropicrin for disinfecting soil in part of a greenhouse bench and also in a number of soil flats. Its effectiveness in these tests led to its application in May, 1941, as a disinfectant in outdoor plant beds, 5×8 ft., in which Colby milo was to be planted. These beds, in which sorghum had been grown for 5 years, had become infested with species of *Pythium*, especially *P. arrhenomanes*.

Chloropicrin was applied May 12 at the rate of 3 cc. per sq. ft. with a special applicator that placed the material 6 in. deep. The soil was then lightly watered and immediately covered with a tarpaulin. After 4 days, the tarpaulin was removed and the soil spaded. On May 29, Colby milo was planted both in the disinfected beds and in similar adjacent beds that also had been watered, covered, and spaded, but had received no chloropicrin.

Emergence was slightly better in the treated soil than in the untreated

[1] Clayton, E. E., J. G. Gaines, G. M. Stone, and K J. Shaw. Soil treatments for tobacco plant beds. (Abstract) Phytopath. **31**: 8. 1941.

[2] Cooke, D. A. The relation of Pythium to growth failure on phosphate-fixing soils. Report of the Assoc. of Hawaiian Sugar Technologists **12**: 169–198. 1933.

[3] Godfrey, G. H. Control of soil fungi by soil fumigation with chloropicrin. Phytopath. **26**: 246–255. 1936.

[4] McLaughlin, J. Harvey, and I. E. Melhus. The response of some field crops on soils treated with chloropicrin. (Abstract) Phytopath. **32**: 15. 1942.

[5] Young, P. A. Soil fumigation with chloropicrin and carbon bisulfide to control tomato root knot and wilt. Phytopath. **30**: 860–865. 1940.

controls, and the disinfected soil was free from weeds, while the untreated soil was soon overrun with them. Pronounced differences in growth of the milo plants were not observed until mid-summer, when the plants in the untreated beds began to show symptoms of the "milo-disease." By August

FIG. 1. Colby milo grown in *Pythium*-infested soil, which was, *A*, left untreated, and, *B*, treated with chloropicrin.

15, the contrast between the plants in the treated and untreated soils was very striking (Fig. 1). Those in the treated soil were vigorous and healthy and were producing excellent heads, whereas those in the untreated soil were stunted and dying and the heads were not filled. *Pythium arrheno-*

manes was readily isolated from the crowns and roots of these diseased plants.

Chloropicrin should not be used in greenhouses in which plants are growing, because growing plants are very sensitive to it. Complete directions for its application may be obtained from the manufacturers.—R. W. LEUKEL, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.